ADVANCES IN AGRONOMY

Contributors

Numbers in Parentheses indicate the pages on which the authors' contributions begin.

N. A. Akram (249)
Department of Botany, University of Agriculture, Faisalabad, Pakistan

F. Al-Qurainy (249)
Department of Botany and Microbiology, King Saud University, Riyadh, Saudi Arabia

M. Ashraf (249)
Department of Botany, University of Agriculture, Faisalabad, Pakistan and Department of Botany and Microbiology, King Saud University, Riyadh, Saudi Arabia

V. C. Baligar (51)
USDA-ARS, Beltsville Agricultural Research Center, Beltsville Maryland, USA

R. B. Clark[1] (51)
USDA-ARS, Appalachian Farming Systems Research Center, Beaver, West Virginia, USA

Francisco Diez-Gonzalez (1)
Department of Food Science and Nutrition, University of Minnesota, St. Paul, Minnesota, USA

M. R. Foolad (249)
Department of Horticulture, The Pennsylvania State University, University Park, Pennsylvania, USA

R. F. Korcak[1] (51)
USDA-ARS, Beltsville, Maryland, USA

P. Krishnan[2] (87)
Crop Systems and Global Change Laboratory, USDA-ARS, BARC West, Beltsville, Maryland, USA and Laboratory of Plant Physiology, Central Rice Research Institute, Cuttack, Orissa, India

Virender Kumar (297)
International Rice Research Institute, India office, Pusa, New Delhi, India

[1] Retired
[2] Present address: Division of Agricultural Physics, Indian Agricultural Research Institute, New Delhi, India

Jagdish K. Ladha (297)
International Rice Research Institute, India office, Pusa, New Delhi, India

Rajendra Prasad (207)
Indian National Science Academy, New Delhi, India

B. Ramakrishnan[3] (87)
Laboratory of Soil Microbiology, Central Rice Research Institute, Cuttack, Orissa, India

K. Raja Reddy (87)
Department of Plant and Soil Sciences, Mississippi State University, Mississippi State, Mississippi, USA

V. R. Reddy (87)
Crop Systems and Global Change Laboratory, USDA-ARS, BARC West, Beltsville, Maryland, USA

Stelios Viazis (1)
Department of Food Science and Nutrition, University of Minnesota, St. Paul, Minnesota, USA

R. J. Wright[1] (51)
USDA-ARS, Beltsville, Maryland, USA

[3] Present address: Division of Microbiology, Indian Agricultural Research Institute, New Delhi, India

Preface

Volume 111 of *Advances in Agronomy* contains six excellent reviews that deal with major global issues: food safety, environmental quality, and food production. Chapter 1 is a comprehensive review of an important food-borne pathogen*, Escherichia coli*. Chapter 2 deals with the application of flue gas desulfurization product to land. Chapter 3 covers the effects of high temperature on rice growth, yield, and grain quality. Chapter 4 is a timely review on aerobic rice systems and includes sections on development of aerobic rice varieties, water saving techniques, and sustainability of aerobic rice systems. Chapter 5 addresses an issue relevant to the increasing concerns about drought conditions around the world—ways to enhance plant drought tolerance. Chapter 6 provides a current assessment of efforts in direct seeding of rice.

I am grateful to the authors for their excellent contributions.

Donald L. Sparks
Newark, Delaware, USA

CHAPTER ONE

Enterohemorrhagic *Escherichia coli*: The Twentieth Century's Emerging Foodborne Pathogen: A Review

Stelios Viazis *and* Francisco Diez-Gonzalez

Contents

Department of Food Science and Nutrition, University of Minnesota, St. Paul, Minnesota, USA

Advances in Agronomy, Volume 111
ISSN 0065-2113, DOI: 10.1016/B978-0-12-387689-8.00006-0

Abstract

Enterohemorrhagic *Escherichia coli* (EHEC) have been recognized as a cause of serious illness and mortality in outbreaks of foodborne illness that involve a large variety of foods. In general, most pathogenic strains behave biochemically and ecologically like any other nonpathogenic *E. coli*, making their detection among commensal *E. coli* an important problem, especially among EHEC. *E. coli* infections in humans are transmitted directly from animals, by person-to-person contact or through contaminated foods. Multiple massive outbreaks associated with the consumption of fresh vegetables have occurred in the past as evidenced by the bagged spinach and lettuce in the United States. There have been numerous studies on pre- and postharvest intervention methods, but the problem is still at large. In the United States and in other countries, the presence of this pathogen in foods is highly regulated and there have been rapid scientific advances in understanding the growth and survival of the pathogen in various foods. This chapter highlights the current understanding of EHEC from the perspectives of food microbiology, molecular microbiology, biochemistry, epidemiology, and agricultural practices with main emphasis on leafy green vegetables. This thesis stresses the importance of developing novel control strategies that are effective and have the potential to be considered natural or organic.

1. Introduction

Enterohemorrhagic *Escherichia coli* (EHEC) have been recognized as a cause of serious illness and mortality in outbreaks of foodborne illness that involve a large variety of foods (Bell, 2002). Generic *E. coli* can be a harmless member of the normal microflora in humans and other animals. However, virulence genes acquired through various means have bestowed different types of pathogenicity to strains of *E. coli*. There are a number of different enteropathogenic groups of *E. coli* that have been shown to cause various types of gastrointestinal infections. Six main pathotypes of *E. coli* can be distinguished: enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), diffusely adhering *E. coli* (DAEC),

enteroaggregative *E. coli* (EAEC), and EHEC. All these pathotypes of *E. coli* use multistep systems of pathogenesis, comprised in general of colonization of the mucosal site, evasion of the host defenses, and multiplication and host damage (Kaper, 2005). After the first outbreak in 1982, *E. coli* O157:H7 has become the most widely known EHEC strain (Riley *et al.*, 1983).

In general, many pathogenic strains behave biochemically and ecologically like any other nonpathogenic *E. coli*, making their detection among commensal *E. coli* an important problem, especially among EHEC (Bettelheim, 2007). Serotype O157 has been found to be unable to ferment the carbohydrate sorbitol (Riley *et al.*, 1983), a phenotypic characteristic that is useful in the organism's detection. Compared to other pathogenic *E. coli*, this serotype would cause hemorrhagic colitis (HC) and other severe symptoms. Other serotypes, such as O26, O111, and sorbitol-fermenting O157:NM, have also been associated with HC and subsequently classified as EHEC (Armstrong *et al.*, 1996). The ability to produce Shiga toxins is the common characteristics of all EHEC that are often referred to as Shiga toxin-producing *E. coli* (STEC).

In this chapter, we examine the versatility of *E. coli* O157:H7 in causing disease through various sources and examine the incidence of infections associated with this pathogen. Potential methods for the control of *E. coli* O157:H7 on a pre- and postharvest level are also discussed. The significance of *E. coli* O157:H7 as a human pathogen stresses the importance of establishing effective strategies to minimize numbers of *E. coli* O157:H7 on the farm.

2. History

E. coli has been recognized as an important human pathogen since its discovery in 1885 by Dr. Theodor Escherich through his work on bacteria in infant stools. The finding of *Shigella dysenteriae* as an agent of epidemic bacterial dysentery by Kioshi Shiga was reported in 1898 (Shiga, 1898). It was in 1955 that hemolytic uremic syndrome (HUS) was first described and defined (Gasser *et al.*, 1955), while Keusch *et al.* (1972) showed that Shiga toxins contribute to bloody diarrhea. Konowalchuk *et al.* (1977) found that certain pathogenic *E. coli* strains produce a toxin capable of killing Vero cells, and in 1982, there were two outbreaks of a severe bloody diarrheal syndrome in Oregon and Michigan associated with the consumption of fast food hamburgers (Riley *et al.*, 1983). O'Brien and LaVeck (1983) reported that an *E. coli* O157:H7 strain that was involved in an outbreak of HC in the United States produced a Shiga toxin, and Karmali *et al.* (1985) suggested that STEC were epidemiologically associated with HUS.

3. Epidemiology

3.1. Outbreaks and incidence

STEC and specifically *E. coli* O157:H7 are considered as emerging food-borne pathogens that occur worldwide but are most common in some parts of the United Kingdom, United States, and Canada (Altekruse *et al.*, 1999; Nataro and Kaper, 1998; Tauxe, 1997). In the United States, *E. coli* O157:H7 is estimated to cause 73,480 illnesses annually, with ~2168 hospitalizations and 61 deaths (Mead *et al.*, 1999), while the pathogen's associated economic costs have been estimated to be 405 million USD (Frenzen *et al.*, 2005). In recent years, EHEC has been the culprit of outbreaks linked to fresh produce (Bell, 2002; De Roever, 1998), with *E. coli* O157:H7 being one of the leading causes of produce-related outbreaks, accounting for 20% of the outbreaks in which the etiological agent was identified (Olsen *et al.*, 2000). Although some of the first outbreaks of *E. coli* O157:H7 were linked to inadequately cooked hamburgers, many outbreaks that followed have been associated with the consumption of raw vegetables, including the massive 1996 outbreak in Japan in which nearly 8000 people were infected by contaminated radish sprouts (Kaper and Karmali, 2008).

Multiple large outbreaks associated with the consumption of fresh vegetables have occurred in the past as evidenced by the radish sprout outbreak in Japan (Michino *et al.*, 1999), bagged spinach and lettuce in the United States (Centers for Disease Control and Prevention, 2006), and fresh lettuce in Sweden (Söderström *et al.*, 2005). In Europe, ~14,000 cases in over 24 countries have occurred from 2000 to 2005, of which 62% belong to the O157 serogroup (Fisher and Meakins, 2006). In England and Wales, salad, vegetables, and fruit caused 6.4% and 10.1% of all outbreaks with a known food vehicle in the periods of 1993–1998 and 1999–2000, respectively (Brandl, 2006).The incidence of foodborne illness associated with the consumption of minimally processed ready-to-eat (RTE) salad vegetables has been consistently increasing (Beuchat, 1998; Kaneko *et al.*, 1999; Tauxe, 1997). Between the years of 1990 and 2001, contaminated fresh produce was associated with a total of 148 outbreaks comprising ~9% of all foodborne outbreaks (Smith DeWaal *et al.*, 2002). Fresh fruits and vegetables are more and more being identified as a source of foodborne outbreaks around the world (Lynch *et al.*, 2009). In the United States, the percentage of outbreaks associated with fresh produce increased from <1% in the 1970s to 6% in the 1990s (Sivapalasingam *et al.*, 2004). The median size of outbreaks associated with fresh produce has doubled and the proportion of outbreak-associated cases related to fresh produce increased from <1% to 12% of illnesses. In Australia, fresh produce was responsible for 4% of all foodborne outbreaks reported between 2001 and 2005 (Kirk *et al.*, 2008).

In 2005, a large outbreak of STEC from lettuce occurred in Sweden and water from a nearby stream was confirmed as positive for Shiga toxin 2 (Stx2) by polymerase chain reaction (PCR; Söderström *et al.*, 2008). Between August and October of 2006, the widely reported multistate outbreak of EHEC occurred in the United States due to contaminated bagged spinach (Calvin, 2007). In December 2006, a STEC outbreak occurred in the Northeastern United States, affecting individuals in New Jersey, New York, and Pennsylvania. The source of the outbreak was traced to iceberg lettuce used at Taco Bell restaurants (Food and Drug Administration, 2006). Also in 2006, Utah and New Mexico health departments investigated a multistate cluster of STEC O157 associated with consuming bagged spinach (Team *et al.*, 2007). Between September and October of 2007, there was an outbreak of STEC O157 in the Netherlands and Iceland. The most probable cause of the outbreak was contaminated lettuce but samples of the product that were tested were all negative for the pathogen (Friesema *et al.*, 2008).

The increase of foodborne outbreaks due to the consumption of fresh vegetables has stressed the importance of developing antimicrobial strategies to reduce their microbial load (Ackers *et al.*, 1998; Calvin, 2007; Hilborn *et al.*, 1999; Johnston *et al.*, 2006; Maki, 2006; Team *et al.*, 2007; Uhlich *et al.*, 2007; Welinder-Olsson *et al.*, 2004). The most recent outbreaks of foodborne disease caused by bagged lettuce and spinach have exposed the limited effect of current conventional washes and the industry as a whole would benefit from research on alternative effective antimicrobial treatments (Calvin, 2007; Maki, 2006).

4. Transmission Vehicles

The primary habitat of *E. coli* is the intestinal tract of warm-blooded animals as well as humans (Bell, 2002). *E. coli* infections in humans are transmitted directly from animals, by person-to-person contact, or through contaminated foods. Enteric pathogens are distributed from livestock to food crops and can occur in various ways such as application of manures, irrigation with contaminated water, dispersal by air, and dispersal via biological vectors, such as wildlife and insects (Janisiewicz *et al.*, 1999). There is widespread fecal contamination of the environment due to farm and wild animals providing a continual source of EHEC in the environment (Bell, 2002). Ground beef is still the most frequently implicated source of *E. coli* O157:H7 outbreaks, accounting for ~75% of *E. coli* O157:H7 outbreaks (Vugia *et al.*, 2006). Dairy products and undercooked minced beef can be directly contaminated by cattle feces during either milking or slaughtering processes (Fremaux *et al.*, 2008).

Talley *et al.* (2009) investigated the role of insects, which frequent fields of leafy greens and neighboring rangeland habitats, in produce contamination. In the study, house flies were confined on manure or agar medium containing *E. coli* O157:H7 tagged with green fluorescent protein (GFP) followed by testing their ability to transfer the pathogen to spinach plants. GFP-tagged bacteria were detected on surfaces of 50–100% of leaves examined by fluorescence microscopy and in 100% of samples tested by PCR. Evidently, flies are capable of contaminating leafy greens, and this confirms the importance of the role of insects in the contamination of fresh produce.

Raw fruit and vegetables, which are indirectly contaminated via irrigation water or through soil treated with farm effluents, are an important vehicle of EHEC contamination (Hilborn *et al.*, 1999; Michino *et al.*, 1999). A recent study on the persistence of *E. coli* O157 in irrigation waters that could potentially be transmitted to fresh produce was conducted in Kubanni River in Nigeria (Chigor *et al.*, 2010). The prevalence of the pathogen in the river was studied over a 10-month period. The detection rate for *E. coli* O157 was 2.1% and fecal coliform counts exceeded acceptable limits. The investigators concluded that the Kubanni River represented a public health risk and unfit for fresh produce irrigation.

The factors responsible for the emergence of the problem are listed in Table 1. A study by Voetsch *et al.* (2006) determined that most STEC O157 infections in 1999–2000 were associated with eating pink hamburgers, drinking untreated surface water, and contact with cattle. Eating produce was inversely associated with infection. Further, direct or indirect contact with cattle waste was a leading identified source of sporadic STEC

Table 1 Factors involved in the emergence of produce-linked outbreaks (Brandl, 2006; Tauxe *et al.*, 1997)

Changes in the produce industry
Intensification and centralization of production
Wider distribution of produce over longer distances
Introduction of minimally processed produce
Increased importation of fresh produce
Changes in consumer habits
Increased consumption of meals outside the home
Increased popularity of salad bars
Increased consumption of fresh fruits and vegetables, and fresh fruit juices
Increased size of at-risk population
Enhanced epidemiological surveillance
Improved methods to identify and track pathogens
Emerging pathogens with low infectious dose

O157 infections. A study by Mukherjee *et al.* (2007) conducted microbiological analyses of fruits and vegetables in Minnesota and Wisconsin farms in conjunction with collecting a farmer survey on farm management practices that may affect the risk of *E. coli* contamination in fresh produce. They found that using animal wastes for fertilization of produce plants posed an increased risk of *E. coli* contamination in organic and semiorganic produce. In addition, improper aging of untreated animal manure also increased the risk in organic produce. It should be noted that organic growers who used cattle manure for fertilization had significantly greater risk compared to those who used other types of manure-based fertilizer. Further, leaf age has also been shown to be a risk factor for O157 contamination (Brandl and Amundson, 2008).

However, commercially grown fresh produce that has been microbiologically tested for pathogenic *E. coli* generally give rise to negative findings, suggesting that contamination with pathogenic *E. coli* is a relatively rare occurrence (Delaquis *et al.*, 2007). A study by Johnston *et al.* (2006) reported that there was no *E. coli* O157:H7 detected in 466 produce and environmental swabs collected in eight packing sheds in the southern United States. Leaf lettuce collected directly from Norwegian farms with organic production practices that included the use of manure fertilizers was also free of *E. coli* O157:H7 (Loncarevic *et al.*, 2005). In addition, *E. coli* O157:H7 and Shiga toxins 1 (Stx1) and 2 were not detected within *E. coli* isolates recovered from organically or conventionally grown produce from Minnesota (Mukherjee *et al.*, 2004). Similarly, no *E. coli* O157:H7 were reported for an expanded investigation that included farms with varied agronomic practices in both Minnesota and Wisconsin (Mukherjee *et al.*, 2006).

5. Microorganism Characteristics

5.1. Unique traits

The genus *E. coli* comprised Gram-negative, facultative anaerobic bacilli, common inhabitant of the gastrointestinal tract of mammals, and belong to the *Enterobacteriaceae* family. They are bile-tolerant, nonfastidious organisms that are easily cultured on routine laboratory media. They ferment lactose and grow best under mesophilic temperatures with an optimum at 37 °C. Most *E. coli* have the β-glucoronidase enzyme that breaks down complex carbohydrates. This enzyme is used in a fluorogenic assay that takes advantage of the breakdown of 4-methyl-umbeliferone glucoronide (MUG) by β-glucoronidase producing a fluorescent compound. However, *E. coli* O157:H7 does not have β-glucoronidase. Further, *E. coli* O157:H7 cannot ferment sorbitol within 24 h, while 90% of *E. coli* can.

The infectious dose of EHEC is very low, between 1 and 100 CFU, which is a much lower dose than for most other pathogens of the intestines (Paton and Paton, 1998a). One of the main characteristics of EHEC that are required to cause disease in humans is their ability to attach to intestinal cells and to colonize the human gut (Welinder-Olsson and Kaijser, 2005).

Since the 1980s, EHEC strains have been established as foodborne pathogens associated with an array of human infections including HC, milder forms of diarrheal illness, and as the major etiologic agent responsible for the fatal infection, HUS. In general, infection with EHEC O157:H7 is self-limiting, but depending on the virulence of the infecting strain, the extent of the disease may vary. HC is the principal disease associated with EHEC and is characterized by severe abdominal cramping and bloody diarrhea. HUS may eventually develop as a sequelae to EHEC infection and HC. HUS is characterized by microangiopathic hemolytic anemia, thrombocytopenia, renal insufficiency or failure, and occasional azotemia (Griffin and Tauxe, 1991; Johnson *et al.*, 2006; Paton and Paton, 1998a). Approximately 8% of those infected with EHEC O157:H7 will develop HUS (McNabb *et al.*, 2008; Tarr, 2009). Diarrhea-associated HUS is largely responsible for morbidity and mortality due to EHEC, resulting in death in up to 5% of infected individuals and frequent permanent renal injury at a rate of 25% (Garg *et al.*, 2003). The endothelial cell damage leads to swollen, detached endothelial cells, which in turn exposes the basement membrane. This leads to platelet activation and local intravascular thrombosis or thrombotic microangiopathy, blood clot formation within the vasculature, and ultimately, a reduction in platelet counts. Hemolytic anemia is an abnormal breakdown of erythrocytes. This results from clots and possible side effects from leukocytes on the erythrocyte cell membranes.

Children less than 5 years of age have a higher incidence of HUS. They express higher levels of the Gb3 receptor present on the renal endothelial cells and form an attachment to Shiga toxin that may be circulating due to infection. Renal injury occurs from leukocyte infiltrates and clots that may lead to acute renal failure and azotemia. Azotemia is characterized by the increase of nitrogenous compounds due to poor filtering by the kidneys (Inward *et al.*, 1997; Tarr, 2009; Tarr *et al.*, 2005). There have been no specific therapeutic regimens developed for *E. coli* O157:H7, but supportive care guidelines have been improved so that mortality rates are low (Tarr *et al.*, 2005).

5.2. Non-O157 EHEC

Non-O157 serotypes have been reported before 1982 and continued to occur on a regular basis in human and animal cases and outbreaks, but very few clinical laboratories actively screen for them, due to lack of reliable detection methods (Bettelheim, 2007). These serotypes lack any obvious

phenotypic characteristic that would allow for differentiation from commensal *E. coli*, and therefore, no standard reliable method exists to isolate, characterize, and identify these pathogens. Unfortunately, they are only found and reported by the most proactive laboratories (Bettelheim, 2007). Even though *E. coli* O157 is reported as the most common EHEC strain in many countries of the world, serotypes O26 and O111 are also recognized as serious threats to public health and have been recovered from patients (Armstrong *et al.*, 1996; Bettelheim, 1996). The lack of isolation methods for non-O157 EHEC has inadvertently led to a lack of awareness for these microorganisms. Based on physiological features, non-O157 EHECs do not possess distinct growth patterns and metabolic characteristics compared to *E. coli* O157:H7 and therefore require confirmation of additional genetic virulence factors (Johnson *et al.*, 1996). However, these methods have the disadvantage of not being able to recover and characterize the identified strains, which is ultimately crucial in tracking non-O157 disease epidemiology and developing strategies for containment (Johnson *et al.*, 1995). In general, isolation methods for non-O157 EHEC still rely on plating on sorbitol MacConkey (SMAC) agar, followed by individual PCR or colony hybridization confirmation for Shiga toxin genes.

The six most common non-O157 STEC associated with disease in the United States have been identified by the CDC as O26, O45, O103, O111, O121, and O145 (Brooks *et al.*, 2005). Ever since non-O157 STEC have become a concern, little data exist for STEC prevalence in ground beef. A recent study reported on the prevalence of STEC on final carcasses and commercial ground beef (Bosilevac and Koohmaraie, 2009). The prevalence of STEC on final carcasses was found to be 3%, while the prevalence of *stx1* and *stx2* genes in ground beef was 26%. The most common STEC was serotype O113. However, other common serotypes, such as O26, O45, O111, and O145, were not isolated from any beef sources. The Minnesota Department of Health conducted a study in which the characteristics of non-O157 and O157 STEC infections identified through sentinel surveillance were compared (Hedican *et al.*, 2009). Overall, the non-O157 STEC isolates recovered from stool specimens obtained from ill patients were found slightly more frequently than O157, and non-O157 cases were identified with the same frequency in the urban and rural populations. O157 infections were found to be more severe, but non-O157 infections caused significant morbidity as well.

5.3. Stress responses

Acid resistance is defined as the ability of bacteria to survive low pH and weak acids (Bearson *et al.*, 1997). Many intestinal bacteria have the ability to survive extreme acid conditions to colonize the host's intestine and survive the low pH gastric environment (Bearson *et al.*, 1997). As many as four stress

response systems capable of protecting stationary-phase cells against acidic environments have been identified in *E. coli* (Lin *et al*., 1995, 1996). When bacteria reach stationary phase, they undergo genetic or morphologic changes allowing them to be more resistant to stresses such as osmotic, heat, cold, or acid shock, compared to log-phase cells (Cheville *et al*., 1996). It has been shown that σ^s is the master regulator of the stationary-phase response and is the product of the *rpoS* gene of *E. coli* (Lange and Hengge-Aronis, 1991). Stress resistance is the result of the expression of proteins on entry into stationary phase that help protect housekeeping and metabolic enzymes from denaturation (Atlung and Ingmer, 1997). In stationary phase, DNA replication is repressed by H-NS proteins, compacting DNA and increasing resistance to various stresses (White, 2000).

Five kinds of decarboxylases have been identified in bacteria in general: glutamate-, arginine-, lysine-, ornithine-, and histamine-decarboxylase (Gale, 1946). Most enteric bacteria are likely to possess at least one of the aforementioned decarboxylases. They function by converting their respective amino acid into carbon dioxide and an amine product while increase the intracellular pH (Gale, 1946). Glutamate, arginine, and lysine decarboxylase have been shown to protect bacteria from pH values between 2 and 3 (Bearson *et al*., 1997).

The oxidative acid resistance mechanism is observed only if cells are grown in mild acidic complex media in the absence of glucose, and once activated, cells can survive a pH of 2.5 or greater in minimal glucose medium (Castanie-Cornet *et al*., 1999). The alternative sigma factor *rpoS* is involved in controlling gene expression while transitioning from log phase to stationary phase, whereas other factors such as cyclic AMP and cAMP receptor protein (CRP) influence the regulation of this system (Castanie-Cornet *et al*., 1999). One of the AR systems requires glutamate for protection at pH 2.5 or lower and involves two glutamate decarboxylase isozymes and the putative glutamate: γ-aminobutyric acid (GABA) antiporter encoded by *gadAB* and *gadC*, respectively (Foster, 2000). The genes encoding GadA and GadB are found at different sites in the chromosome, 78 and 33 min, respectively, and they share a 98% homologous sequence and encode glutamate decarboxylase isozymes (De Biase *et al*., 1999).

The glutamic acid decarboxylase (GAD) enzymes are pyridoxyl phosphate-containing enzymes that replace the alpha-carboxyl groups of their amino acid substrates with a proton that is taken up from the cytoplasm, while carbon dioxide and GABA are produced as the end products (Foster, 2004). Studies conducted by Foster (Foster, 2004) show that once an AR system has been induced, *E. coli* O157:H7 can remain acid resistant for at least a month during refrigeration. The ability of *E. coli* to survive acid exposure depends on the growth stage that they reach before acid challenge (Large *et al*., 2005). In addition, after exposure to acidic condition, the survival rate is significantly lower for log-phase cells compared to

stationary-phase cells under the same conditions (Foster, 2004; Large *et al*., 2005). This is because AR systems are controlled in part by the RpoS alternative sigma factor that is activated in stationary phase and in turn activates and represses proteins required for the response to various stresses (Large *et al*., 2005; Merrell and Camilli, 2002). It has been shown that *rpoS* mutants are more acid sensitive and show an inability to utilize arginine and glutamate AR systems (Lin *et al*., 1996). Stationary-phase cells may be more AR compared to exponentially growing cells, but it is commonly accepted that they can become AR.

Some genes expressed in stationary phase can cause the same gene expression as when *E. coli* is preexposed to physical stress and can ultimately lead to protection from other stresses also known as cross protection (Cheville *et al*., 1996; Johnson, 2003). The *gadA*, *gadBC*, and the regulatory *gadE* gene are induced by low pH in exponential phase cells grown in minimal glucose media (Ma *et al*., 2004). Under normal conditions when *E. coli* is growing in rich media, expression of most decarboxylase systems is repressed by the CRP, and it is relieved in minimal media for glutamate decarboxylase (Diez-Gonzalez and Karaibrahimoglu, 2004). Acidic anaerobic media have been found to induce AR in *E. coli* cells, while a number of genetic systems are known to be coinduced by acid and anaerobiosis (Small *et al*., 1994). Adding $CdCl_2$ and ethanol to log-phase *E. coli* W3110 has been shown to inhibit growth by 50% and shift the growth curve to stationary phase (VanBogelen *et al*., 1987), during which cells are most AR. The induction of *gadA* through exposure to *N*-acyl-L-homoserine lactone increased acid tolerance (pH 4) of *E. coli* in log phase (Houdt *et al*., 2006). Neutralized medium filtrates from *E. coli* grown to stationary phase at pH 5 induce acid tolerance in log-phase cells growing at pH 7 (Rowbury and Goodson, 1998). It has been suggested that *E. coli* expels one or more compounds during growth to stationary phase that can confer a protection against AS in log phase (Mates *et al*., 2007).

GadAB/C genes are induced in response to stationary-phase signals, while known regulators that affect their expression include RpoS, GadW, and GadX (De Biase *et al*., 1999). The level of involvement of each regulator apparently depends on the growth phase and the medium. Any acid resistance induction that can withstand low pH levels in exponential phase in minimal glucose media has been demonstrated to depend on the activation of GadAB/C that results primarily from the induction of GadE (Ma *et al*., 2004).

5.4. Virulence factors

The pathogenicity of STEC is determined by several virulence factors that are encoded by chromosomal pathogenicity islands, phage chromosomes integrated in the bacterial genome as well as plasmids. Virulence factors that

appear necessary for virulence of EHEC O157 are the Shiga toxins, the locus for enterocyte effacement (LEE), and the large plasmid pO157.

5.5. Shiga toxins

Shiga toxins are members of a toxin family that share many common features. The Shiga toxins identified in EHEC are classified in two distinct subgroups: Stx1 and Stx2 (O'Brien and Holmes, 1987). The toxins are produced by the pathogen in the colon and cause local damage as well as have the ability to travel through the bloodstream to the kidney where it is thought to play a role in causing HC and HUS (Kaper, 2005). Common characteristics of Shiga toxins include the fact that both toxins are polymeric consisting of an A subunit and pentameric B subunits. Both are encoded in an operon with the A subunit gene proximal to the B subunit gene. Further, they are both phage and chromosomally encoded (Endo *et al.*, 1987, 1988). The B subunit mediates binding to receptors in eukaryotic cell membranes, while the A units have *N*-glycosidase activity, which lead to cell death by inhibition of protein synthesis at the level of 28S ribosomal RNA. Stx2 is 1000 times more cytotoxic than Stx1 (Endo *et al.*, 1987, 1988). The toxin phenotypes are variable among non-O157 STEC, while there is considerable epidemiological evidence that suggests that STEC isolates producing Stx2 are more commonly associated with serious disease compared to isolates producing only Stx1 (Barbieri *et al.*, 1999).

5.6. Attaching and effacing adherence

A whole cluster of virulence factors encoded by a chromosomal region called the LEE is present in many STEC strains and are responsible for attaching and effacing lesions. The LEE encodes for a type II secretion factor, an adhesion called intimin (*eaeA*), and for the translocated receptor of intimin (Nataro and Kaper, 1998). Intimin is a 94- to 97-kDa outer membrane protein that has been identified in EHEC (Albert, 1992). Intimin is the only bacterial adherence factor identified in EHEC for intestinal colonization in an animal model (Kaper *et al.*, 1998). The ability of STEC to produce attaching effacing lesions is sufficient to cause nonbloody diarrhea but Shiga toxin is essential for the development of bloody diarrhea, HC, and HUS (Nataro and Kaper, 1998). The host epithelial receptor for intimin, also known as the translocated intimin receptor (Tir), is encoded by LEE and is secreted for efficient delivery into the host cell (DeVinney *et al.*, 1999).

Other structures that assist EHEC in adhering to host cells are fimbriae and fimbrial adhesins, thread-like structures that extend out from the bacterial surface. Type 1 fimbriae are the first adhesins described in *E. coli* (Duguid *et al.*, 1955) and are the most common adhesins produced.

These adhesins promote the adherence of the pathogen to mannose-containing glycoproteins found on the surfaces of eukaryotic cells. P fimbriae recognize a disaccharide of uroepithelial cells and human erythrocytes (Leffler and Svanborg-Eden, 1981), while S fimbriae mediate adherence to endothelial cells (Parkkinen *et al.*, 1989). F1845 is one of four adhesins that belong to the Dr family of adhesins (Nowicki *et al.*, 1990) and the only one associated with diarrheagenic *E. coli*.

5.7. The pO157 plasmid

Plasmid pO157 is ~90 kb in size and has been found in almost all EHEC O157 isolates. The plasmid contains EHEC-hemolysin that is cytotoxic for human and bovine cell lines. EHEC strains also produce two different types of enterohemolysins. Plasmid encoding enterohemolysin genes are found in the 60-MDa EHEC plasmid, which when expressed can cause a hemolytic phenotype on washed sheep blood agar with small zones of lysis (Schmidt *et al.*, 1995). There is also a bacteriophage-associated enterohemolysin that has been identified as serologically and genetically distinct from the plasmid-encoded hemolysin and only the plasmid-encoding hemolysin is typically associated with EHEC pathogenicity (Schmidt *et al.*, 1995). Another pO157-encoded virulence factor includes EHEC KatP catalase–peroxidase, which is produced in addition to the two chromosomally encoded catalases or hydroperoxidases of *E. coli* (Brunder *et al.*, 1996). Catalases assist the bacterium against oxidative stress, while peroxidases are heme-binding enzymes that carry out a variety of functions (Loewen *et al.*, 1985).

5.8. EHEC virulence profile

EHEC clinical isolates from HUS patients have been found to have a distinct virulence profile. Strains capable of producing both Shiga toxins have been found to be highly associated with bloody diarrhea or HUS, while strains with only Stx1 are rarely found in HUS patients (Law, 2000). In addition, clinical strains associated with HUS have also been found to be more enterohemolytic and are more likely to possess intimin (Law, 2000). The high virulence of STEC strains like *E. coli* O157:H7 is not only dependent on virulence factors but partially also on the pathogen's ability to survive environmental stress conditions, such as resistance to low pH levels found in the gastrointestinal tract, contributing to its very low infectious dose of 50–100 cells or lower (Armstrong *et al.*, 1996). It has been shown that the combined presence of *stx2* and *eae* genes is an important predictor of HUS (Ethelberg *et al.*, 2004). From a clinical standpoint, when a patient has been infected with *E. coli* and has developed HUS symptoms, then STEC tend to be classified as EHEC (Meng *et al.*, 2001). A summary of the virulence found in EHEC/STEC isolates can be found in Table 2.

Table 2 Possible and known virulence factors in EHEC/STEC isolates of human and cattle origin (Welinder-Olsson and Kaijser, 2005)

Virulence factors	Gene	P/C	Proven/possible importance
Verocytotoxin 1	*VT1*	C	Inhibits protein synthesis, causing cell death
Verocytotoxin 2	*VT2*	C	
Intimin	*eaeA*	C	Mediates intimate adherence to epithelial cells
EHEC-hemolysin	*E-hly*	P	Hemolytic to washed sheep RBC
Type II secretion	*etpD*	P	Secretes extracellular proteins
Catalase–peroxidase	*katP*	P	Defense against oxidative stress, etc.
Serine protease	*espP*	P	Cleaves coagulation factor V, etc.
Type 1 fimbriae	*fimA*	C	Adhesins that enable adherence to host cells
P fimbriae	*papC*	C	
S fimbriae	*sfaD/sfaE*	C	
F 1845	*daaE*	C	
Aerobactin	*iutA*	C	Mediates bacterial iron acquisition
α-Hemolysin	*hlyA*	C	Osmotic lysis of target cells

P, plasmid; C, chromosomal.

5.9. Isolation

According to the USDA's Food Safety and Inspection Service (FSIS), ground beef is considered adulterated if as little as 1 CFU of EHEC O157:H7 is detected in 25 g of ground beef. *E. coli* O157:H7 is the only EHEC for which an official isolation method is in place. There are three types of methods used for STEC detection, and most of them depend on Shiga toxin gene detection. Cell culture cytotoxicity assays are conducted with vero cells, allowing for screening of clinical samples, but resulting in many false negatives when food samples are processed. Immunological detection methods are dependent on antibodies that bind to antigens such as Shiga toxins of O somatic antigens of cells. These methods can produce presumptive results in a short period of time. DNA-based methods such as PCR amplification can be used to screen different types of STEC with the primer-targeting Shiga toxin genes (Ramotar *et al.*, 1995; Yavzori *et al.*, 1998). Currently, a number of multiplex-PCR assays have been developed for the various virulence genes enterohemolysin (*hly*), intimin (*eae*), and the two Shiga toxins (*stx1, stx2*; Fagan *et al.*, 1999; Paton and Paton, 1998b). Since these three virulence factors can be detected simultaneously, this allows for specific detection of EHEC.

Natural feces generally have low levels of *E. coli* O157:H7, and enrichment steps are required to increase the levels of the organism to

allow for detection. Enteric bacteria have similar physiological characteristics and therefore enrichment may cause the outgrowth of competitive microflora as well. To improve selective growth of *E. coli* O157:H7, selective media have been developed through the addition of antibiotics (Heuvelink *et al.*, 1997). There are three types of enrichment media that are often used when recovering *E. coli* O157:H7: buffered peptone water supplemented with 8 mg/L vancomycin, 10 mg/L cefsulodin, and 0.05 mg/L cefixime, modified EC broth (mEC with novobiocin), or mTSB with 20 mg/L novobiocin or 10 mg/L acriflavin (OIE, 2006).

Strains of *E. coli* O157 are relatively easy to isolate because of their unique biochemical characteristics. STEC O157 is unable to ferment the carbohydrate sorbitol, which led to the development of the SMAC agar (March and Ratnam, 1986) used for its isolation. More specific media have also been developed, such as Rainbow Agar, CHROMagar®, and O157:H7 ID agar, that are able to recover STEC O157 along with sorbitol-fermenting O157 and non-O157 strains (Bettelheim, 2007). Other methods have also been developed to differentiate and identify STEC O157 and can be also used in various ways to identify non-O157 strains. Non-O157 serotypes can be differentiated from commensal *E. coli* by using specialized molecular techniques such as multiplex PCR (Fagan *et al.*, 1999). Immunomagnetic beads have been designed for the capture of the O antigen of O157 and some have been developed for the most commonly reported non-O157 strains such as O111 and O26 (Oxoid, Inc.). SMAC containing cefixime and tellurite (CT-SMAC) provides highly selective recovery of *E. coli* O157:H7 from other *E. coli* and enteric bacteria. Currently, CT-SMAC is widely used to isolate *E. coli* O157:H7 followed by PCR or latex agglutination confirmation. However, the use of CT-SMAC is not recommended for detection of non-O157 EHEC because most non-O157 EHECs that produce Shiga toxins behave physiologically the same as other commensal *E. coli* strains (Arthur *et al.*, 2002; Johnson *et al.*, 1996). Recently, a 16-plex PCR was developed, taking advantage of the multitude virulence factors found in diarrheagenic *E. coli*, including EHEC, EIEC, EAEC, EPEC, and ETEC (Antikainen *et al.*, 2009). The virulence factors used for EHEC included *stx1*, *stx2*, *eae*, *escV*, *ent*, and *hly*, while the specificity of the PCR was 100% when tested with 289 control strains.

6. Ecology and Evolution

6.1. Microbial ecology

6.1.1. Animals

Past studies have shown that as many as 30% of cattle can be asymptomatic carriers of *E. coli* O157:H7 (Reinstein *et al.*, 2007; Stanford *et al.*, 2005). Further, manure from cattle feedlots and production facilities may contain

viable *E. coli* O157:H7 and can be subsequently washed into the water supply. Then it may be consumed directly in drinking water, or be used as irrigation water on crops, or transmitted by other animal vectors (Hill *et al.*, 2006; LeJeune *et al.*, 2001; Sargeant *et al.*, 2003). To this end, interventions that help reduce *E. coli* O157:H7 populations in food animals before they enter the food chain have great potential to reduce human illnesses (Callaway *et al.*, 2007). Studies have shown that EHEC O157:H7 are carried through the bovine gastrointestinal tract and recently have been shown to be highly colonized and associated with the terminal rectal mucosa (Nart *et al.*, 2008; Naylor *et al.*, 2003). There is an increase in the amount of EHEC O157:H7 shed in cattle feces during the summer months, which correlates with increases in illness. In fact, in 2006, the CDC reported that 43.47% of all STEC infections occurred in July, August, and September (McNabb *et al.*, 2008). Further, carriage and shedding of EHEC has been reported to be more frequent with heifers and calves that generally show longer periods of shedding than older cattle (Armstrong *et al.*, 1996).

A survey conducted by Cobbold *et al.* 2008 compared non-O157 Shiga toxigenic *E. coli* recovered from bovine, human, raw milk, and beef. The distribution of Shiga toxin genes among isolates indicated that *stx1* was predominant in milk, *stx2* on carcasses, and the combination of both *stx1* and *stx2* in beef. They found that the virulence factors *eae* and *hly* were found at 23% and 15% of isolates, respectively. These findings highlight the importance of non-O157 as a threat to health since they are commonly found in food, and warrants critical assessment.

6.1.2. Environment

Some cattle are referred to as "super-shedders" and excrete EHEC for extended periods of time at levels as high as 10^4 CFU/g (Matthews *et al.*, 2006). The shedding of EHEC in the environment by cattle increases the probability of other cattle harboring the pathogen through contamination of feed, water, fecal contamination of their hides, and other routes. These factors contribute to the almost ubiquitous presence of EHEC in any environment near cattle. Fresh fruit and vegetables may become contaminated with EHEC at various stages of their production. Contact with the soil, use of improperly composted manure, contaminated irrigation water, poor personnel hygiene, poor sanitation of equipment, and wild animals could contribute to the dissemination of EHEC onto the field (Beuchat, 2006; Franz *et al.*, 2008b; Todd *et al.*, 2009).

Contamination of vegetables grown in soils that have been enriched with contaminated manure depends on how well the pathogen survives in manure and manure-amended soils (Franz and van Bruggen, 2008). The survival of enteric pathogens decreases once excreted from the animal gut (Unc and Goss, 2004). *E. coli* O157 has been reported to be able to survive

for long periods of time in manure (Franz *et al*., 2005; Scott *et al*., 2006) and manure-amended soil (Franz *et al*., 2008a). Contamination of crops can occur through one of three ways: uptake via the root system, splash dispersal from the soil surface, or directly by irrigation water (Franz and van Bruggen, 2008). Several studies demonstrated the association of *E. coli* O157:H7 and *Salmonella* with vegetable surfaces when grown in soils enriched with contaminated manure (Islam *et al*., 2004a,b), while the potential internal presence of pathogens is of concern since these cells will most likely not be removed by postharvest or consumer sanitation actions, thereby posing a serious public health threat.

Recent work by plant pathologists and food microbiologists suggests that the connections between foodborne pathogens and fresh produce are more complicated than simple passive transfer (Tyler and Triplett, 2008). The exact mechanism of contamination in the majority of produce-related outbreaks is usually unexplained, but research following the outbreaks suggests a highly complex ecology of the environment. Outbreak-related and other strains of *E. coli* O157:H7 have been routinely isolated from water sources in and around the area found to be the likely source of several lettuce-related *E. coli* O157:H7 outbreaks (Cooley *et al*., 2007).

Harvesting and processing of fresh produce can cause plant tissue damage (Barker-Reid *et al*., 2009). A recent study attempted to assess the role of plant tissue damage in relation to contamination of leafy greens with *E. coli* O157:H7 (Brandl, 2008). The effect of mechanical, physiological, and plant disease-induced lesions on the growth of the pathogen on postharvest romaine lettuce was investigated. Only 4 h after inoculation, concentrations of *E. coli* O157:H7 increased 4.0-, 4.5-, and 11.0-fold on lettuce leaves that were mechanically bruised, cut into large pieces, and shredded into multiple pieces, respectively. *E. coli* O157:H7 concentrations increased only twofold on leaves that were left intact after harvest. In addition, the concentration of *E. coli* O157:H7 was 27 times greater on young leaves affected by soft rot due to infection by *Erwinia chrysanthemi* compared to healthy middle-aged leaves. It would be suggested that growers postpone contaminated water irrigation of lettuce crops with suspected injuries for a minimum of 2 days or use the highest microbiological quality of water available (Barker-Reid *et al*., 2009).

EHEC O157 and non-O157 have been shown to adhere to the leaf surface of spinach and lettuce through the use of EspA filaments, which play a major role in colonization of human and bovine hosts (Dziva *et al*., 2007; Shaw *et al*., 2008). A study by Shaw *et al*. showed that O157 and non-O157 EHEC use a filamentous type III secretion system (fT3SS) for colonization of lettuce leaves (Shaw *et al*., 2008). In contrast to the colonization of the mammalian host, EHEC adhesion to leaves is independent of effector protein translocation. EHEC do not cause disease once introduced into the inter-mesophyl cellular space but instead act as opportunistic epiphytes using the plant as a transmission vector (Shaw *et al*., 2008).

6.1.3. Molecular evolution

Evolutionary analysis through genome sequencing has shown that O157:H7 strains are genetically closely related to enteropathogenic *E. coli* O55:H7 strains (Feng *et al.*, 1998). A model has been developed that shows that O157:H7 has evolved to its current form through a series of transitional steps from a nontoxigenic ancestor (Feng *et al.*, 1998). The most common ancestor of O157:H7 and O55:H7 strains contained the LEE that allowed the strain to cause diarrhea through an attachment-effacement mechanism (Wick *et al.*, 2005). Further, this common ancestor had the ability to ferment sorbitol and express β-glucoronidase, thus resembling wild-type *E. coli* (Wick *et al.*, 2005). One of the first evolutionary steps included the acquisition of Stx2 hypothetically through transduction, followed by the large virulence plasmid (pO157), leading to the change of somatic antigen from O55 to O157 (Wick *et al.*, 2005). The genes encoding Stx are encoded in the genome of heterogeneous lambdoid prophages. Stx-phages represent highly mobile genetic elements that play an important role in the expression of Stx, horizontal gene transfer, and genome diversification (Herold *et al.*, 2004). From that early STEC, two distinct lines emerged. The first line lost motility through a mutation in the flagellar operon, which lead to the sorbitol-fermenting O157 clone that has been found in HUS cases in Germany (Karch and Bielaszewska, 2001; Monday *et al.*, 2004). The second line lost the ability to ferment sorbitol and acquired Stx1. Consequently, the *uidA* gene was inactivated through mutation, giving rise to the non-sorbitol-fermenting, β-glucoronidase-negative phenotype of the frequently occurring serotype O157:H7.

There have been several outbreaks involving O157 contamination of fresh produce, such as lettuce and spinach that were associated with more severe disease. These outbreaks lead to higher frequencies of HUS and hospitalization and suggested that increased virulence has evolved (Manning *et al.*, 2008). This hypothesis was tested through phylogenetic analyses that identified 39 SNP genotypes and were separated into nine distinct clades. There were significant differences observed between the different clades regarding their frequency and distribution of Shiga toxin genes as well as the type and severity of clinical disease they caused. It was found that patients with HUS were more likely to be infected with clade 8 strains (Manning *et al.*, 2008). The frequency of clade 8 strains has increased from 10% in 2002 to 46% in 2006 despite a decrease in total O157 cases identified in this time period (Manning *et al.*, 2008).

A study by Ogura *et al.* (2009) compared the genomes of EHEC serotypes O157, O26, O111, and O103. The investigators demonstrated how *E. coli* strains of different phylogenies can independently evolve into EHEC strains. Specifically, this study determined that the EHEC genomes contained many lambdoid phages, integrative elements, and virulence

plasmids that contained the same or similar virulence genes but apparently had distinct evolutionary histories. This indicated that independent acquisition of these mobile genetic elements had driven the evolution of each particular EHEC. Of interest is the evolution of the type III secretion system (T3SS). The study found that the T3SS of EHEC is composed of genes that were introduced by three different types of genetic elements: (a) an integrative element referred to as the locus of enterocyte effacement, which encodes a central part of the T3SS; (b) SpLE3-like integrative elements; and (c) lambdoid phages carrying numerous T3SS effector genes and other T3SS-related genes. In addition to gains and losses of phage elements, O157:H7 genomes are rapidly diverging and radiating into new niches as the pathogen disseminates (Wick *et al.*, 2005).

E coli O157:H7 strains can be classified into different genotypes based on the presence of specific Shiga toxin-encoding bacteriophage insertion sites. Some genotypes are highly associated with human clinical cases, while others are more frequently found in bovine cases (Besser *et al.*, 2007). A recent study compared the expression patterns of clinical genotype 1 strains with those of bovine-biased genotype 5 strains using microarrays (Vanaja *et al.*, 2010). What they found was that virulence factors, such as LEE genes, enterohemolysin, and pO157 genes, were highly expressed in clinical-biased genotypes. Further, genes essential for acid resistance and stress fitness were upregulated in bovine-biased genotypes.

7. Methods of Control

7.1. Postharvest interventions

Some varieties of fresh-cut fruits and vegetables are no longer considered low risk in terms of food safety (Bhagwat, 2006). One criticism of many of the investigations for sanitizing treatments for fresh produce is that they are used in extreme doses, excessive washing times sometimes unauthorized substances (Gil *et al.*, 2009).

7.2. Temperature

Thermal processing is one of the most common interventions applied to foods to inactivate EHEC (Erickson and Doyle, 2007). The heat sensitivity of the pathogen has been extensively studied and reviewed. In recent years, mild heat treatments and high temperature short time treatments have been evaluated to inactivate STEC on raw produce and meat. However, a new study raised an important question. Pasteurization temperatures have been validated for STEC, but not for free Shiga toxin (Rasooly and Do, 2010). The investigators measured Shiga toxin's inhibition effect on Vero cell

dehydrogenase activity and protein synthesis after treatment with pasteurization temperatures. Shiga toxin 2 was found to be heat stable and that pasteurization of milk, as suggested by the U.S. Food and Drug Administration (63 °C for 30 min, or 72 °C for 15 s or 89 °C for 1 s), did not reduce the biological activity of Stx2. However, treatment at 100 °C for 5 min inactivated the toxin.

A recent study investigated the effect of temperature on the survival and growth of EHEC on prewashed, chopped romaine lettuce (CRL) and grated iceberg lettuce (GIL) (Sudarshana *et al.*, 2008). It was found that on CRL held at 4 °C, the EHEC population decreased by 2 log CFU/g after 5 days. At 20 °C, the population increased by one log within 24 h and remained constant thereafter. In GIL, the EHEC population increased by >1 log CFU/g after 24 h and continued to increase throughout the 5-day study period. Storage at 20 °C for 48 h resulted in deterioration of quality which was more in GIL.

7.3. High pressure

The application of high pressure processing (HHP) to enhance the safety of seeds or sprouts has been studied in the past (Ariefdjohan *et al.*, 2004; Penas *et al.*, 2008) and various degrees of efficacy have been shown. A recent study showed that the application of HHP on sprouts inoculated with EHEC O157:H7 can lead to more than a 5 log CFU/g reduction after 15 min at 650 MPa at 20 °C (Neetoo *et al.*, 2008).

7.4. Ultrasound

Power ultrasound, as used for cleaning in the electronics industry, has a potential to be used as an application to decontaminate fresh produce (Seymour *et al.*, 2002). In a recent study conducted by Zhou *et al.* (2009), ultrasound was used in combination with chlorine, acidified sodium chlorite, peroxyacetic acid, acidic electrolyzed water, and on its own to inactivate EHEC O157:H7 spot inoculated on spinach leaves. Ultrasonication significantly enhanced the reduction of the pathogen for all treatments by 0.7–1.1 log cycles over that of each sanitizer on its own. The best combination of treatments was ultrasonication and acidified sodium chlorite (200 mg/L), resulting in a 4 log CFU inactivation.

7.5. Ionizing irradiation

Food irradiation uses high-energy gamma rays, electron beams, or X-rays; all are penetrating processes and are used commercially to eliminate pathogens from meat products (Smith and Pillai, 2004). Irradiation may be better than most technologies in penetrating fresh produce and it could be a

powerful tool if used correctly in different produce items and among different varieties. Irradiation is able to effectively eliminate *E. coli* O157:H7 from lettuce (Niemira *et al.*, 2002). Niemira compared the antimicrobial efficacy of sodium hypochlorite washes and ionizing radiation for the elimination of leaf-internalized *E. coli* O157:H7 (Niemira, 2007). The study found that 300 and 600 ppm sodium hypochlorite resulted in less than 1 log reduction in the pathogen. However, when ionizing radiation was used at 1.5 kGy, the pathogen was reduced by 4 log CFU in romaine lettuce and 3 log CFU in spinach. Another study showed that irradiation up to 1.0 kGy can result in 3–4 log CFU reduction of internalized *E. coli* on lettuce leaves (Gomes *et al.*, 2009).

X-ray is a nonthermal technology that has potential for reducing pathogens on spinach leaves. A recent study found that more than a 5 log CFU reduction/leaf can be achieved with 2.0 kGy X-ray for *E. coli* O157:H7 (Mahmoud *et al.*, 2009).

7.6. Ozone

Ozone destroys microorganisms through progressive oxidation of critical cellular components, with the cell surface suggested as the primary target of the process. Chlorine, one of the most commonly used disinfecting agents, destroys certain intracellular enzyme systems, while ozone causes widespread oxidation of internal cellular proteins ultimately leading to rapid cell death (Komanapalli and Lau, 1996). When apples inoculated with *E. coli* O157:H7 are treated with bubbling ozone during washing or through dipping, there was a 3.7 and 2.6 log CFU decrease, respectively (Achen and Yousef, 2001).

7.7. Ultraviolet light

Ultraviolet (UV) light is a type of nonionizing radiation with wavelengths from 100 to 400 nm. Radiation has been used both to delay ripening-associated processes and to reduce microorganism growth (Vicente *et al.*, 2005). When pulsed UV light was applied to strawberries, there were maximum reductions of *E. coli* O157:H7 of 2.1 log CFU/g at 25.7 J/cm^2, on raspberries 3.9 log CFU/g at 72 J/cm^2, and on blueberries 2.9 log CFU/g at 1.27 J/cm^2. There was no observable damage to the fruits at these UV doses (Bialka and Demirci, 2007, 2008).

7.8. Radio frequency

A study by Nelson *et al.* (2002) evaluated radio frequencies (RF) as a method for reducing *Salmonella*, *E. coli* O157:H7, and *Listeria monocytogenes* contamination in alfalfa seeds. Short RF exposures produced reductions in

the target organisms without adverse effect on seed germination. However, it was found that by extending RF exposure to produce the desired level of microbial reduction, there was an adverse effect on germination.

7.9. Chemical antimicrobials

Sanitizers that can be used to wash fruits and vegetables are regulated by the U.S. Food and Drug Administration in accordance with the Federal Food, Drug, and Cosmetic Act as outlined in the Code of Federal Regulations, Title 21, Ch. 1, Section 173.315. One of the few treatments commonly used by large distributors of fresh produce is washing, and this procedure is often enhanced by including a sanitizing agent in the washing water. Use of a disinfectant can enhance efficiency of removal up to 100-fold, but chemical treatments administered to whole and cut produce typically will not reduce populations of pathogens by more than 2–3 log CFU/g (Beuchat, 2009). Chlorine is used under widely varying postharvest procedures (Beuchat and Ryu, 1997). Little specific information is available on chlorine dosages and contact times to achieve maximum inactivation of produce-associated microbes. In general, the chlorine dosages (50–200 ppm) and contact times (1–2 min) used by produce processors generally result in 1–2 log (90–99%) bacterial inactivation (Casteel *et al.*, 2008).

Chlorine is also used in the form of sodium hypochlorite; less commonly used are citric and ascorbic acid (1%), and there have been a number of recent commercially available products based on synthetic sanitizers (Francis *et al.*, 1999). Past studies have made a strong case for the shortcomings of chlorine as an effective antimicrobial. It has been shown that sometimes it is as effective as, or marginally more effective than, deionized water in removing *E. coli* O157:H7 from the lettuce leaf and is unable to kill pathogens within damaged portions of the leaf or pathogens that have infiltrated into the leaf tissue (Beuchat, 1999; Li *et al.*, 2008; Takeuchi and Frank, 2000). Past research suggests that the surface structure of lettuce protects *E. coli* O157:H7 from inactivation by chlorine (Takeuchi and Frank, 2000).

7.10. Cinnamaldehyde

There has been an increased interest in the development and application of new effective and nontoxic antimicrobial compounds. Plant essential oils (EOs) have been found to have antimicrobial activity against a multitude of pathogens and show promise as an alternative to the currently used sanitizers (Friedman *et al.*, 2002). Plant-derived EOs can be used as flavoring agents in foods and beverages and have potential as natural agents for food preservation due to their content of antimicrobial compounds (Helander *et al.*, 1998).

Cinnamon oil is commonly used in the food industry because of its special aroma. Further, its antimicrobial activity has also attracted great attention from many researchers.

Cinnamaldehyde is present in numerous commercial foods at concentrations of up to 0.03% (Friedman *et al*., 2000) and is listed as generally accepted as safe by the Flavor and Extract Manufacturers Association (Adams *et al*., 2004). *Cinnamomum cassia* bark oil is used in food and beverages and has high value from a commerce perspective. The main components of *C. cassia* bark oil are cinnamaldehyde and coumarin (Hu *et al*., 1985). These two compounds are used as food additives. It has also been demonstrated that *Cinnamomum zeylanicum* oil can inhibit meat spoilage organisms (Ouattara *et al*., 1997).

When assessing the antimicrobial action of EO components, the lipophilic character of their hydrocarbon skeleton and the hydrophilic character of their functional groups are of the main importance. The activity rank of EO components is as follows: phenols > aldehydes > ketones > alcohols > ethers > hydrocarbons. A study by Helander *et al.* (1998) used different EOs to inhibit *E. coli* O157:H7 and *Salmonella* and they found that *trans*-cinnamaldehyde gains access to the periplasm and to the deeper parts of the cell, yet does not result in the disintegration of the outer membrane or deplete the intracellular ATP pool. Another study found that the minimum inhibitory concentration of cinnamaldehyde against *E. coli* was 500 μg/mL and its high antimicrobial activity was attributed to its aldehyde group, while a conjugated double bond, a long CH chain outside the ring, and the hydroxyl group may also be responsible (Chang *et al*., 2001). In addition, the carbonyl group is thought to bind to proteins, preventing the action of amino acid decarboxylases in *E. aerogenes* (Wendakoon and Sakaguchi, 1995). Di Pasqua *et al.* (2006) used fatty acid extraction and gas chromatographic analysis to assess changes in membrane fatty acid composition of *E. coli* treated with *trans*-cinnamaldehyde. Substantial changes were observed on the long chain unsaturated fatty acids when the *E. coli* strains grew in the presence of limonene and cinnamaldehyde.

Yang *et al.* (2010) evaluated the effects of supplementing the diet of feedlot cattle with cinnamaldehyde on intake, growth performance, carcass characteristics, and blood metabolites. They found that including cinnamaldehyde in the diet of feedlot cattle, particularly early in the feeding period, may help promote intake and reduce the effects of stress. Charles *et al.* (2008) investigated the potential of low concentrations of *trans*-cinnamaldehyde to inactivate *E. coli* O157:H7 in cattle drinking water. All *trans*-cinnamaldehyde concentrations used effectively inactivated *E. coli* O157:H7 in water and the magnitude of killing significantly increased *trans*-cinnamaldehyde concentrations increased as well as increases in storage temperature. The presence of feed or feces in water significantly decreased the antibacterial effect of *trans*-cinnamaldehyde on *E. coli* O157:H7.

It may be possible to use EOs in foods not previously associated with a herby or spicy flavor if the presence of one or more synergists can produce the desired antibacterial effect at a concentration which does not produce undesirable changes in the flavor or aroma (Burt, 2004). Baskaran *et al.* (2010) investigated the antimicrobial effect of low concentrations of *trans*-cinnamaldehyde on *E. coli* O157:H7 in apple juice and apple cider. They found that at 4 °C, 0.125% and 0.075% (v/v) cinnamaldehyde decreased the pathogen counts in the juice and cider to undetectable levels on days 3 and 5, respectively. These results showed that low concentrations of cinnamaldehyde could be used as an effective antimicrobial to inactivate *E. coli* O157:H7 in apple juice and apple cider.

A study by Juneja and Friedman (2008) tested the heat resistance of a four-strain mixture of *E. coli* O157:H7 in raw ground beef in the presence of cinnamaldehyde. They found that contaminated sous vide-processed ground beef should be heated to an internal temperature of 60 °C for at least 30.3 min to achieve a 4D reduction. Cinnamaldehyde and thymol are effective against six *Salmonella* serotypes on alfalfa seeds when applied in hot air at 50 °C as fumigation. Increasing the temperature to 70 °C reduced the effectiveness of the treatment (Weissinger *et al.*, 2001). An active component of allspice, eugenol, has been found to have a suppressive effect on the production of intracellular and extracellular Shiga toxins by stationary-phase *E. coli* O157:H7 (Takemasa *et al.*, 2009). Antimicrobials in the vapor phase might be more effective in inactivating *E. coli* O157:H7 cells attached to leafy greens than aqueous antimicrobials.

EO can also be used against hospital-acquired infection in humans, specifically uropathogenic attached to urinary catheters as biofilms. Recently, Amalaradjou *et al.* (2010) treated polystyrene plates and urinary catheters inoculated with uropathogenic *E. coli* (5–6.0 log CFU) with difference concentrations of *trans*-cinnamaldehyde at 37 °C. They found that all concentrations of the antimicrobial resulted in effectively preventing the pathogen from forming a biofilm on plates and catheters, while producing no cytotoxic effects on human bladder epithelial cells.

7.11. Electrochemically activated water

Electrochemically activated water (EAW) has been reported to have strong bactericidal effects on most pathogenic bacteria that are important to food safety (Huang *et al.*, 2008). EAW is produced by passing a diluted salt solution through an electrolytic cell that contains an anode and cathode separated by a membrane. By subjecting the electrodes to direct current voltages, negatively charged ions such as chloride and hydroxide in the diluted salt solution move to the anode and become oxygen gas, chlorine gas, hypochlorite ion, hypochlorous acid, and hydrochloric acid, while

positively charged ions move to the cathode to take up electrons becoming hydrogen gas and sodium hydroxide (Hsu, 2005). The main advantage of EAW is its safety. EAW which is also a strong acid is different to hydrochloric acid or sulfuric acid in that it is not corrosive to skin, mucous membrane, or organic material. Koseki *et al.* (2004) used mildly heated (50 °C) EAW to treat lettuce for 5 min, followed by cold (4 °C) EAW water to treat for 1 or 5 min. They found the treatment could reduce both *E. coli* O157:H7 and *Salmonella* at a level of 3–4 log CFU/g. Pangloli *et al.* (2009) evaluated the efficacy of electrolyzed water in killing *E. coli* O157:H7 on iceberg lettuce through the use of washing and/or chilling treatments simulating those followed in food service kitchens. They found that the greatest reduction levels on lettuce were achieved by sequentially washing with 14-A acidic electrolyzed water for 15 or 30 s followed by chilling in 16-A acidic electrolyzed water for 15 min. This procedure reduced the pathogen by 2.8 and 3.0 log CFU per leaf, respectively. A study by Keskinen *et al.* (2009) compared the efficacy of chlorine (20–200 ppm), acidic electrolyzed water (50 ppm chlorine, pH 2.6), acidified sodium chlorite (20–200 ppm chlorite ion concentration, Sanova®), and aqueous chlorine dioxide (20–200 ppm chlorite ion concentration, Trinova®) washes in reducing populations of *E. coli* O157:H7 on lettuce. They found that the chlorite ion solutions were the most effective against *E. coli* O157:H7 populations on iceberg lettuce, with log reductions as high as 1.25 and 1.05 log CFU/g for TriNova® and Sanova® wash treatments, respectively. In contrast to previous studies, the acidic electrolyzed water as well as the rest of the treatments resulted in reductions of less than 1 log CFU/g on iceberg lettuce. Chlorine (200 ppm), TriNova®, Sanova®, and acidic electrolyzed water were all equally effective against *E. coli* O157:H7 on romaine, with log reductions of ~1 log CFU/g.

7.12. Bacteriophages

Bacteriophages are viruses that prey on bacteria, offering a natural nonantibiotic method to reduce pathogens from the food supply (Callaway *et al.*, 2008). *E. coli* phages can be isolated from sewage, hospital waste water, polluted rivers, and fecal samples of humans or animals (Brussow, 2005). The metabolic state of the bacterial host is crucial for bacteriophage infection and propagation, since adsorption, replication, lytic activity, and survival of the phage are affected (Farrah, 1987; Williams *et al.*, 1999). A number of culture experiments have demonstrated that optimal proliferation and yield of phages are observed at ideal growth conditions of the host (Lenski, 1988). Host generation times can influence phage latent periods (Guixa-Boixareu *et al.*, 1996; Middelboe, 2000) and low nutrient availability may result in increased latent periods and reduced burst sizes (Middelboe, 2000; Proctor *et al.*, 1993) suggest that phage propagation

depends on host metabolism significantly. Stationary-phase cells may allow phage maturation to proceed, but cell lysis can be stopped (Middelboe, 2000).

It is highly recommended to create cocktails of several phages to obtain sufficient breadth of host range and to reduce the probability of phage resistance (Brussow, 2005). Phages utilized for pathogen control in foods and food production systems usually originate from environmental samples and other nonfood sources such as municipal waste water, feces, sewage, soil, farms, and processing facility effluents (Connerton *et al.*, 2004; Dykes and Moorhead, 2002; O'Flynn *et al.*, 2004; Pao *et al.*, 2004). Phages have also been suggested as a possible treatment strategy for dealing various bacterial infections and have been demonstrated to be effective against urinary tract infections in mice (Nishikawa *et al.*, 2008) and respiratory infections in chickens (Huff *et al.*, 2003). In a large study, stool samples from 600 healthy patients and 140 patients suffering from traveler's diarrhea were investigated for the presence of coliphages on 10 different *E. coli* strains (Furuse, 1987). From healthy subjects, 34% of the stool samples contained phages but only 1% showed high amounts. Further, most of them were temperate phages. However, 70% of the stools from diarrhea patients contained phages, of which 18% were in high concentrations. Coliphages can be used as a surrogate measure for fecal contamination of recreational waters or other waters of public health interest (el-Abagy *et al.*, 1988).

Using specific phages to eliminate or reduce the levels of contaminated bacteria on fresh-cut fruits and vegetables is also noted to be under investigation for *E. coli* O157:H7 (Kudva *et al.*, 1999). As part of an ongoing study, Sharma *et al.* (2009) tested the effectiveness of a mixture of bacteriophages in reducing *E. coli* O157:H7 gfp 86 on cut pieces of iceberg lettuce and cantaloupe. They found that the bacteriophage treatment reduced the pathogen immediately upon application to lettuce and the bacteriophage treatments had significant lower counts of the pathogen for both the lettuce and the cantaloupe compared to the negative control. A study by Niu *et al.* (2009) evaluated the host range and lytic capability of four phages against *E. coli* O157 from cattle and humans. They found that the phages were effective against the majority of the bovine and human STEC O157 isolates and suggested that lytic capability and host range should be considered when selecting a therapeutic phage for on-farm control of STEC O157. Further, they advocated for the use of phage cocktails as an effective mitigating approach for STEC O157 due to the observation that some STEC O157 isolates exhibited resistance to some but not all phages.

Bacteria frequently live in biofilms, which are surface-associated communities encased in a hydrated extracellular polymeric substance (EPS) matrix that is composed of polysaccharides, proteins, nucleic acids, and lipids and helps maintain a complex heterogeneous structure (Davey and O'toole, 2000; Xavier *et al.*, 2005). Bacterial biofilms have been implicated

as a source of infection and contamination in medical, industrial, and food processing settings because of their resistance to antimicrobial agents and host defenses (Xavier *et al.*, 2005). There is a growing need for novel and effective treatments for biofilms due to their increasingly apparent antibiotic resistance and the fact that antibiotics can even induce biofilm formation (Stewart and William Costerton, 2001).

Bacteriophages have been proposed as a method for controlling biofilms in several studies. Sharma *et al.* (2005) used an alkaline cleaner and a bacteriophage to treat *E. coli* O157:H7 in biofilms on stainless steel and found that even though populations of cells that were attached on coupons were reduced by the phage, the cells enmeshed in biofilms were protected. Corbin *et al.* (2001) found that biofilms under carbon limitation can act as natural reservoirs for bacteriophage and that the phages can have some influence on biofilm morphology. In a study by Tait *et al.* (2002), bacteriophages specific for *Enterobacter* strains were isolated from primary effluent sewage. Combinations of three phages were required to completely eradicate biofilms of *Enterobacter cloace*. However, when trying to eliminate a susceptible bacterial population within a dual species biofilm, the attempt was unsuccessful. This suggested that phages would be a poor tool by themselves for controlling biofilm formations, but a combined treatment with a disinfectant may be successful.

Research by Lu and Collins (2007) showed that dispersing biofilms of *E. coli* was possible at 4.5 orders of magnitude through the use of engineered enzymatic bacteriophages. The investigators engineered a bacteriophage to express a biofilm-degrading enzyme that was capable of attacking the bacterial cells in the biofilm and the biofilm matrix itself. The enzyme, Dispersin B (DspB), is produced by *Actinobacillus actinoinyceteincomitans*, and is able to hydrolyze β-1,6-*N*-acetyl-D-glucosamine, which is an adhesin needed for biofilm formation and integrity in *Staphylococcus* and *E. coli* (Hughes *et al.*, 1998). Though not a "natural" antimicrobial, engineered bacteriophages that produce polysaccharide depolymerases can reduce bacterial biofilms by attacking both the biofilm collectively and the bacteria individually. Reports of natural lytic phages with phage-borne polysaccharide depolymerases have shown that phage-induced lysis and EPS degradation can be used in combination in natural systems to reduce biofilms (Hughes *et al.*, 1998).

Advantages of using phages over traditional antimicrobial systems for foods have been reviewed on the pre- (Barrow and Soothill, 1997; Joerger, 2003) and postharvest level (Leverentz *et al.*, 2001, 2003). Phages are highly specific and their use in agriculture is not likely to select for phage resistance in untargeted bacterial species. Further, bacterial resistance mechanisms against phages and antibiotics differ, thus the possible emergence of resistance against phages will not affect the susceptibility of bacteria to antibiotics used for humans. In addition, phage preparations can readily be modified in

response to changes in bacterial pathogen populations or susceptibility, while antibiotics have a long and expensive development cycle (Sulakvelidze and Barrow, 2005). In addition, recently there has been exploration in different phage delivery systems. Puapermpoonsiri *et al.* (2009) showed that phages specific for *Staphylococcus aureus* or *Pseudomonas aeruginosa* could be encapsulated into biodegradable polyester microspheres via a modified w/o/w double emulsion-solvent extraction protocol resulting in only a partial loss of lytic activity. Despite the poor shelf life of the formulation, the work is proof of concept for the formulation and controlled delivery of bacteriophages, as acceptable for the treatment of bacterial lung infections.

Using combined treatments is consistent with the hurdle concept (Leistner, 1992), which states that effective control of foodborne pathogens can be achieved through the use of a combination of compatible control measures to ensure the safety of food. The phage treatment is a new and effective hurdle, which in combination with *trans*-cinnamaldehyde and/or other control measures may maximize protection from foodborne pathogens on vegetables.

EOs such as *trans*-cinnamaldehyde (Juneja and Friedman, 2008; Weissinger *et al.*, 2001; Yang *et al.*, 2010) have been successfully applied to suppress the activity of phytopathogens. Ye *et al.* have used a combination of *Enterobacter asburiae* JX1 and a cocktail of five lytic bacteriophages to evaluate their efficacy against *Salmonella* Javiana on tomatoes (Ye *et al.*, 2009) and sprouting mung beans and alfalfa seeds (Ye *et al.*, 2010). They found that the combination was successful for the sprouting mung beans and alfalfa seeds; however, there was no evidence to suggest that the antagonistic activity of *E. asburiae* could be enhanced with phages when used on tomatoes. Leverentz *et al.* (2003) applied phages in combination with nisin against *L. monocytogenes* on fresh-cut honeydew melons and fresh-cut apples. They found that the phages on their own inactivated *L. monocytogenes* by 2.0–4.6 log CFU over the control, while nisin on its own resulted in a 3.2 log CFU reduction on melons. However, when the two treatments were used in combination, there was a 5.7 log CFU inactivation. On the other hand, the synergy between the phages and nisin that was exhibited on melons was not demonstrated on fresh-cut apples. Roy *et al.* (1993) used a combination of *L. monocytogenes*-specific phages and QUATAL, a quaternary ammonium compound to disinfect *L. monocytogenes* from stainless steel and polypropylene surfaces. A synergistic activity was observed when the phages were suspended in QUATAL and found that the phages were not affected by the compound at 50 ppm and a contact time of 4 h.

In another example of using a phage with an additional antimicrobial, Huff *et al.* (2004) used a bacteriophage and Baytril (enrofloxacin) to treat colibacillosis in broiler chicken. Mortality in the birds was 3% when treated with enrofloxacin and 15% when treated with the phage alone. However,

the birds that received both treatments had total protection. A recent study by Los *et al.* (2008) suggested that when isolated phages from the environment, the use of sublethal concentrations of antibiotics could result in an increase in plaque diameters and isolation of phages which could have been overlooked due to formation of small plaques or no plaques at all. The success of the method can be attributed to a partial imbalance in the regulation of lysis inhibition in the host with an impaired, but not fully suppressed, protein synthesis system. The synergy between these two treatments suggested that phages combined with an antibiotic or other antimicrobial, preferably a natural one, have significant value.

7.13. Preharvest interventions

Human illnesses caused by the most common foodborne pathogens cost the U.S. economy alone more than $7 billion each year (Buzby, 2001). Successful strategies to control the prevalence of *E. coli* O157:H7 in ruminants can potentially reduce the threat this pathogen poses. Intervention/supplementation strategies can be grouped into three approaches: competitive enhancement strategies, direct antipathogen strategies, and animal management strategies, of which some are available now and some will be available in the future (Callaway *et al.*, 2007). It should be noted that currently, no reliable intervention or animal vaccine is commercially available. Probiotics used to create an intestinal environment that can inhibit *E. coli* O157:H7 have been tested, but without consistent success (Lema *et al.*, 2001; Ogawa *et al.*, 2001). Hay feeding has been shown to reduce colonization of *E. coli* O157:H7 and decrease prevalence from 52% to 18%, but this effect has not been consistently observed by other researchers (Callaway and Diez-Gonzalez, 2002). An estimated 50–70% of antibiotics used in the United States are given to farm animals (Gustafson, 1991) for three main reasons: (1) prophylactically, to prevent disease in flocks and herds; (2) to treat sick livestock; and (3) to improve digestion and utilization of feed, often resulting to improved weight gain (Sulakvelidze and Barrow, 2005). The use of antibiotics in livestock has become a major source of concern due to the possibility of contributing declining efficacy of antibiotics used to treat bacterial infections to humans (Smith *et al.*, 2002). Bacteria have many complex mechanisms to resist antibiotics, and the widespread use of antibiotics in both human medicine and animal agriculture has led to the broad dissemination of antibiotic resistance genes (Busz *et al.*, 2002; Phillips, 1998). Due to the concern over antibiotic resistance, it is likely that the prophylactic use of antibiotics to promote growth in food animals will become even more highly regulated, or perhaps even prohibited (Callaway *et al.*, 2007). Banning or reducing the application of antibiotics may pose a risk in the safety of foods and the treatment of sick animals, unless an effective, safe, and environmentally friendly alternative is

developed such as the use of bacteriophage-based antibacterial products (Sulakvelidze and Barrow, 2005).

Using bacteriophages to reduce contamination of foods with various pathogens requires in-depth understanding of the epidemiology of the pathogen against which the phage preparation will be used (Stone, 2002). There are three areas of application of phage technology: (1) phages may be used to reduce intestinal colonization of live animals that carry pathogens, (2) phages may be applied directly onto raw foods or onto environmental surfaces in raw food processing facilities, and (3) phages may be applied directly on RTE food (Sulakvelidze and Barrow, 2005).

Phages have been evaluated to control pathogens in a variety of foods of animal origin. Phages of *E. coli* O157:H7 have been characterized and their antibacterial activities have been determined *in vitro* in broths (Kudva *et al.*, 1999; O'Flynn *et al.*, 2004; Ronner and Cliver, 1990). Bacteriophage CEV1 is a phage that specifically infects *E. coli* O157:H7 and was isolated from sheep resistant to colonization by the pathogen (Raya *et al.*, 2006). Sheep that received a single oral dose of the phage showed a 2-log-unit reduction in intestinal *E. coli* O157:H7 levels within 2 days compared to levels in controls. A study conducted by the same research group (Oot *et al.*, 2007) showed that the prevalence of O157:H7-infecting phages in livestock may be grossly underestimated if an enrichment method is not used. In this study, fecal samples from commercial beef feedlot were screened to detect O157:H7-infecting phages, and after an initial screen which produced no recovery of phages, an enrichment protocol was used. This resulted in detection of phages for O157:H7 or nonpathogenic *E. coli* in the majority (97%) of the samples. Jensen *et al.* (1998) suggested that a multiple-host enrichment protocol may be more effective for the isolation of broad-host-range bacteriophages by avoiding the selection bias that single-host methods typically have.

Tanji *et al.* (2004) used three phages to rapidly evacuate *E. coli* O157:H7 in artificially inoculated mice, but the difference of *E. coli* concentration in the feces of mice in the group with phage became slight after the 9-day test period compared to the control group. Tanji *et al.* (2004) demonstrated the effective use of phage cocktails to avoid the emergence of phage-resistant cells. Barrow *et al.* (1998) used an *E. coli*-specific bacteriophage to prevent septicemia in chickens. The control group had a mortality rate of 100% after inoculation with 10^6 CFU, while a single injection of the phage preparation prior to the bacterial challenge prevented morbidity and death. The higher the dose of the phage, the more effective the protection it provided. Similarly, colostrums-deprived calves were challenged with the same strain of *E. coli* leading to septicemic disease, but when calves were injected with the phage preparation, they remained healthy. Despite the statistical limitations of the study, the phages did have a positive therapeutic effect. Huff *et al.* (2002) studied the ability of phage therapy to prevent fatal *E. coli*

respiratory infections in broiler chickens by challenging the groups of 3-day-old chickens with mixtures of the pathogen and phages. They also made 1-week-old chickens drink water with a certain pathogen/phage mix. In the first experiment, mortality rates decreased significantly, from 80% to 5%, while in the second they did not.

A cocktail of phages reduced O157:H7 populations in the feces of sheep by 24 h after treatment (Callaway *et al.*, 2008). Further, a 1:1 ratio of plaque forming units over colony forming units was found to be more effective than higher ratios of 10:1 or 100:1. Another cocktail of three bacteriophages was used by O'Flynn *et al.* (2004) for biocontrol of *E. coli* O157:H7. Bacteriophage-insensitive mutants (BIMs) were recovered at a very low frequency (10^{-6}) and reverted to phage sensitivity after 50 generations. In a meat trial experiment, the phage cocktail completely eliminated *E. coli* O157:H7 from the beef meat surface in seven of nine cases (O'Flynn *et al.*, 2004). Sheng *et al.* (2006) have argued that efforts to consistently clear *E. coli* O157:H7 from cattle may be unrealistic and their study showed that phage therapy would be effective at reducing the levels of intestinal *E. coli* O157:H7 in ruminants, but also highlighted the difficulties in developing an effective phage intervention. In their study, Sheng *et al.* (2006) found that phage therapy reduced the average number of *E. coli* O157:H7 CFU among phage-treated streers compared to the control group but did not eliminate the bacteria from the majority of steers.

A study by Rozema *et al.* (2009) compared the effects of oral and rectal administration of O157-specific phages aimed at reducing the fecal shedding of *STEC* O157. They found that orally treated steers produced the fewest STEC O157 culture-positive samples compared with rectally treated steers and a combination of orally and rectally phage-treated steers. However, this number was barely lower than that for the untreated steers. It is worth noting that phages were isolated from untreated steers, indicating that these specific steers had acquired phages from the environment and shed them at a level similar to that of rectally treated steers. Constant phage therapy has been shown to be an effective method for reducing the shedding of *E. coli* O157:H7 in cattle, as long as the host bacterium is not resistant to phages (Rozema *et al.*, 2009).

Ionophores, such as monensin and lasalocid, are regularly included in the majority of feedlot and dairy rations and are intended to inhibit gram-positive bacteria, resulting in an improved feed to gain ratio and production efficiency (Callaway *et al.*, 2003). It was hypothesized that due to their gram-negative membrane physiology, EHEC would not be affected by these feed additive antimicrobials, giving them a competitive advantage with regard to their role in colonization and shedding (Callaway *et al.*, 2009). However, it was found that ionophoric feed additives (monensin, lasalocid, laidlomycin, and bambermycin) had no effect on *E. coli* O157:H7 *in vitro* (Edrington *et al.*, 2003). Several studies have investigated the

relationship between these feed additive antimicrobials, and many have suggested there is a potential interaction between diet type and antimicrobial treatment, but no definitive proof of this linkage has been demonstrated (Dargatz *et al.*, 1997; Herriott *et al.*, 1998; McAllister *et al.*, 2006).

Neomycin is another antimicrobial that has proved to be effective against EHEC both in ruminants' guts and hides, but it is also used in human medicine and there is concern for antimicrobial resistance (Callaway *et al.*, 2009). A nonantibiotic alternative for reducing the prevalence of *E. coli* O157:H7 includes the use of sodium chlorate by applying it to cattle feed and water (Callaway *et al.*, 2002). Hide washing involves physical removal of contaminants from the hide and hooves from cattle and can significantly reduce carcass contamination (Bosilevac *et al.*, 2005a). Other approaches include using ozonated or electrolyzed water (Bosilevac *et al.*, 2005b).

Vaccination is used to prevent pathogen colonization and fecal excretion in ruminants, and it is based on inducing the animal's immune system to protect itself from antigens expressed by *E. coli* O157:H7 (LeJeune and Wetzel, 2007). Priming the mucosal immune system to have a protective response against an organism that is usually commensal is a difficult task, but researchers have created vaccines targeted against cellular components and proteins that help the organism adhere to the intestinal mucosa of calves (LeJeune and Wetzel, 2007). These include type III proteins, Tir, intimin, and the O157 lipopolysaccharide (Bettelheim, 2003; Konadu *et al.*, 1999; Potter *et al.*, 2004). In a recent clinical vaccine trial, commercially fed cattle were used to test the effect of a two-dose regimen of a vaccine against type III secreted proteins of *E. coli* O157:H7 (Smith *et al.*, 2008). The study found that pens of vaccinated cattle were less likely to test positive for *E. coli* O157:H7. Another study tested the efficacy of a siderophore receptor and porin proteins-based vaccine on *E. coli* O157:H7 in feedlot cattle (Thomson *et al.*, 2009). The investigators found that the prevalence of *E. coli* O157:H7 was lower in vaccinated compared to control animals and vaccination was associated with a 98.2% reduction in *E. coli* O157:H7 concentration in fecal samples.

Feed management has been suggested as a viable method to affect conditions within ruminant gastrointestinal tracts and ultimately modify the survival of *E. coli* O157:H7 (LeJeune and Wetzel, 2007). There have been some conflicting studies on various feedstuffs, and interpretations of results do not always agree between different research groups. For example, early studies suggested that cottonseed and clover feeding could reduce fecal excretion of *E. coli* O157:H7 in dairy cattle (Dargatz *et al.*, 1997), while later studies actually reported a positive association between the two feeds and the prevalence of the pathogen (Sargeant *et al.*, 2004). Corn silage, barley, and beet pulp have been found to increase the prevalence of O157 in cattle (Berg *et al.*, 2004; Dargatz *et al.*, 1997). There is a plethora of explanations on how a specific feed influences the gastrointestinal microflora

such as altering volatile fatty acid concentrations, changing the pH conditions, and altering the composition of the resident bacteria (LeJeune and Wetzel, 2007).

A *Lactobacillus acidophilus* culture has demonstrated effectiveness at reducing *E. coli* O157:H7 in feedlot cattle by up to 50% (Younts-Dahl *et al.*, 2005). This particular product is currently available commercially in the United States and is being used in many large U.S. feedlots (Callaway *et al.*, 2004). Molecules released by probiotic strain La-5 influence the transcription of EHEC genes involved in colonization of epithelial cells (Medellin-Pena and Griffiths, 2009). Further, these molecules are able to prevent the adherence of EHEC to epithelial cells and its capacity to concentrate F-actin at adhesion sites.

Natural microflora present on fresh produce may help reduce the pathogen load. A recent study isolated natural microflora from fresh-cut iceberg lettuce and baby spinach and found them to be antagonistic toward *E. coli* O157:H7 (Johnston *et al.*, 2009). Samples were collected under conditions that mimicked actual practices between production and retail sale. The inhibitory activity by several isolates was due to either acid production or antimicrobial peptides. The most common isolates obtained from multiple processing and storage steps were members of the genera *Pantoea*, *Pseudomonas*, *Klebsiella*, *Enterobacter*, *Aeromonas*, and *Burkholderia*. Cooley *et al.* (2006) investigated the interaction between *E. coli* O157:H7and epiphytic bacteria in lettuce extracts and on inoculated seedlings. Coinoculation with *E. asburiae* was found to reduce survival, while *Wausteria paucula* was found to increase it. These observations suggest that species-specific competitive or commensal relationships likely occur in natural systems (Delaquis *et al.*, 2007).

Lately, due to increased ethanol production, there has been an increased availability of distillers grains, an ethanol fermentation coproduct derived from corn and included in cattle diets as a protein and energy source (Klopfenstein *et al.*, 2007). Recently, there have also been several reports showing evidence that by including distillers grains solids (DGS) in cattle feed leads to an increase of the level of fecal shedding and prevalence of *E. coli* O157:H7 in cattle. Dewell *et al.* (2005) showed that feeding DGS was a risk factor for *E. coli* O157:H7 carriage in beef cattle. An increased fecal prevalence of this pathogen was also observed by another study when animals were fed 30% and 40% DGS in their ratios (Peterson *et al.*, 2007). Other work that was done included 379 naturally infected animals and showed that the prevalence of *E. coli* O157 positive pen samples was 2.5-fold larger in cattle fed 25% distillers grains compared to control samples (Jacob *et al.*, 2008a). The same investigators observed significantly higher levels of *E. coli* O157:H7 in feces and intestinal tissues of animals fed 25% DGS compared with animals fed steam flaked corn (Jacob *et al.*, 2008b). However, in their latest study, Jacob *et al.* (2009b) did not report a

significant difference in the prevalence of naturally infected animals fed DGS or dry-rolled corn.

Bosilevac *et al.* (2009) examined *E. coli* O157:H7 prevalence in feedlot steers that were fed diets with or without wet distillers grains with solubles (WDGS). They found that the average percentage of fecal *E. coli* O157:H7 during the finishing phase for WDGS fed cattle was 2.7% compared to 0.1% for corn fed control cattle. Also, there was no significant difference in the average percentage of *E. coli* O157:H7 hide samples between diets, but the WDGS fed cattle had higher levels. Overall, feeding 40% WDGS may increase the level and prevalence of *E. coli* O157:H7 in feedlot cattle. However, the magnitude of the difference detected in this study could possibly have been skewed by the low prevalence in control cattle. The overall mechanism responsible for the phenomenon of increased *E. coli* O157 prevalence with increased feeding of DGS in cattle is not known (Jacob *et al.*, 2009a). There are two proposed general mechanisms for this trend; distillers grains alter the hindgut ecology of cattle, making the environment suitable for *E. coli* O157, or a component of distillers grains stimulates the growth of the pathogen O157 (Jacob *et al.*, 2008a). Hindgut ecology has been shown to change when cattle are fed distillers grains and at the same time distillers grains have been shown to alter rumen microbial populations (Fron *et al.*, 1996). Further research is required before the mechanism can be elucidated.

8. Outlook

Currently, *E. coli* O157:H7 outbreaks and product recalls as a result of *E. coli* O157:H7 contamination comprise one of the largest threats to the long-term sustainability of the fresh produce industry. Outbreaks such as the lettuce and spinach outbreaks of 2006 undermine consumer confidence and target the fresh produce industry as unable to protect their product and consumers and the beef cattle industry as the source of environmental contamination. To solve this problem, proactive steps must be taken to develop effective strategies capable of reducing fecal shedding of the pathogen by feedlot cattle and to reduce its prevalence and persistence in the environment. Evaluation of preharvest control measures that effectively reduce fecal shedding of *E. coli* O157 by cattle and other ruminants is crucial prior to developing on-farm strategies. These steps could potentially reduce the number of *E. coli* O157:H7-positive animals and thus minimize foodborne illness associated with this pathogen. Effective approaches must start at the farm level before sending cattle to slaughter and follow through with proper measures during growth, harvesting, and packing of fresh produce.

REFERENCES

Achen, M., and Yousef, A. (2001). Efficacy of ozone against *Escherichia coli* O157:H7 on apples. *J. Food Sci.* **66,** 1380–1384.

Ackers, M. L., Mahon, B. E., Leahy, E., Goode, B., Damrow, T., Hayes, P. S., Bibb, W. F., Rice, D. H., Barrett, T. J., Hutwagner, L., Griffin, P. M., and Slutsker, L. (1998). An outbreak of *Escherichia coli* O157:H7 infections associated with leaf lettuce consumption. *J. Infect. Dis.* **177,** 1588–1593.

Adams, T. B., Cohen, S. M., Doull, J., Feron, V. J., Goodman, J. I., Marnett, L. J., Munro, I. C., and Portoghese, P. S. (2004). The FEMA GRAS assessment of cinnamyl derivatives used as flavor ingredients. *Food Chem. Toxicol.* **42,** 157–185.

Albert, M. (1992). Sharing of virulence-associated properties at the phenotypic and genetic levels between enteropathogenic *Escherichia coli* and *Hafnia alvei*. *J. Med. Microbiol.* **37,** 310–314.

Altekruse, S. F., Stern, N. J., Fields, P. I., and Swerdlow, D. L. (1999). Campylobacter jejuni—An emerging foodborne pathogen. *Emerg. Infect. Dis.* **5,** 28–35.

Amalaradjou, M. A. R., Narayanan, A., Baskaran, S. A., and Venkitanarayanan, K. (2010). Antibiofilm effect of trans-cinnamaldehyde on uropathogenic *Escherichia coli*. *J. Urol.* **184** (1), 358–363.

Antikainen, J., Tarkka, E., Haukka, K., Siitonen, A., Vaara, M., and Kirveskari, J. (2009). New 16-plex PCR method for rapid detection of diarrheagenic *Escherichia coli* directly from stool samples. *Eur. J. Clin. Microbiol. Infect. Dis.* **28,** 899–908.

Ariefdjohan, M., Nelson, P., Singh, R., Bhunia, A., Balasubramaniam, V., and Singh, N. (2004). Efficacy of high hydrostatic pressure treatment in reducing *Escherichia coli* O157 and Listeria monocytogenes in alfalfa seeds. *J. Food Sci.* **69,** 117–120.

Armstrong, G. L., Hollingsworth, J., and Morris, J. G. Jr. (1996). Emerging foodborne pathogens: *Escherichia coli* O157:H7 as a model of entry of a new pathogen into the food supply of the developed world. *Epidemiol. Rev.* **18,** 29–51.

Arthur, T. M., Barkocy-Gallagher, G. A., Rivera-Betancourt, M., and Koohmaraie, M. (2002). Prevalence and characterization of non-O157 Shiga toxin-producing *Escherichia coli* on carcasses in commercial beef cattle processing plants. *Appl. Environ. Microbiol.* **68,** 4847–4852.

Atlung, T., and Ingmer, H. (1997). H-NS: A modulator of environmentally regulated gene expression. *Mol. Microbiol.* **24,** 7–17.

Barbieri, J., Wagner, P. L., Acheson, D. W. K., and Waldor, M. K. (1999). Isogenic Lysogens of diverse Shiga toxin 2-encoding bacteriophages produce markedly different amounts of Shiga toxin. *Infect. Immun.* **67,** 6710–6714.

Barker-Reid, F., Harapas, D., Engleitner, S., Kreidl, S., Holmes, R., and Faggian, R. (2009). Persistence of *Escherichia coli* on injured iceberg lettuce in the field, overhead irrigated with contaminated water. *J. Food Prot.* **72,** 458–464.

Barrow, P. A., and Soothill, J. S. (1997). Bacteriophage therapy and prophylaxis: Rediscovery and renewed assessment of potential. *Trends Microbiol.* **5,** 268–271.

Barrow, P., Lovell, M., and Berchieri, A. (1998). Use of lytic bacteriophage for control of experimental *Escherichia coli* septicemia and meningitis in chickens and calves. *Clin. Vaccine Immunol.* **5,** 294–298.

Baskaran, S. A., Amalaradjou, M. A. R., Hoagland, T., and Venkitanarayanan, K. (2010). Inactivation of *Escherichia coli* O157: H7 in apple juice and apple cider by trans-cinnamaldehyde. *Int. J. Food Microbiol.* **141,** 126–129.

Bearson, S., Bearson, B., and Foster, J. W. (1997). Acid stress responses in enterobacteria. *FEMS Microbiol. Lett.* **147,** 173–180.

Bell, C. (2002). Approach to the control of entero-haemorrhagic *Escherichia coli* (EHEC). *Int. J. Food Microbiol.* **78,** 197–216.

Berg, J., McAllister, T., Bach, S., Stilborn, R., Hancock, D., and LeJeune, J. (2004). *Escherichia coli* O157: H7 excretion by commercial feedlot cattle fed either barley-or corn-based finishing diets. *J. Food Prot.* **67,** 666–671.

Besser, T. E., Shaikh, N., Holt, N. J., Tarr, P. I., Konkel, M. E., Malik-Kale, P., Walsh, C. W., Whittam, T. S., and Bono, J. L. (2007). Greater diversity of Shiga toxin-encoding bacteriophage insertion sites among *Escherichia coli* O157: H7 isolates from cattle than in those from humans. *Appl. Environ. Microbiol.* **73,** 671.

Bettelheim, K. (1996). Enterohaemorrhagic *Escherichia coli*—A review. *Int. Food Hyg.* **7,** 5–9.

Bettelheim, K. (2003). Non-O157 verotoxin-producing *Escherichia coli*: A problem, paradox, and paradigm. *Exp. Biol. Med.* **228,** 333–344.

Bettelheim, K. A. (2007). The non-O157 shiga-toxigenic (verocytotoxigenic) *Escherichia coli*; under-rated pathogens. *Crit. Rev. Microbiol.* **33,** 67–87.

Beuchat, L. R. (1999). Survival of enterohemorrhagic *Escherichia coli* O157: H7 in bovine feces applied to lettuce and the effectiveness of chlorinated water as a disinfectant. *J. Food Prot.* **62,** 845–849.

Beuchat, L. R. (2006). Vectors and conditions for preharvest contamination of fruits and vegetables with pathogens capable of causing enteric diseases. *Br. Food J.* **108,** 38–53.

Beuchat, L. R. (1998). Surface decontamination of fruits and vegetables eaten raw: A review. Food safety unit, World Health Organization. Geneva. Switzerland. WHO/FSF/FOS/98.2.

Beuchat, L. R., and Ryu, J. H. (1997). Produce handling and processing practices. *Emerg. Infect. Dis.* **3,** 459–465.

Bhagwat, A. (2006). Microbiological Safety of Fresh-Cut Produce: Where Are We Now. American Society for Microbiology Press, Washington, DC.

Bialka, K., and Demirci, A. (2007). Decontamination of *Escherichia coli* O157: H7 and *Salmonella enterica* on blueberries using ozone and pulsed UV-light. *J. Food Sci.* **72,** 391–395.

Bialka, K., and Demirci, A. (2008). Efficacy of pulsed UV-light for the decontamination of *Escherichia coli* O157: H7 and *Salmonella* spp. on raspberries and strawberries. *J. Food Sci.* **73,** 201–206.

Bosilevac, J., and Koohmaraie, M. F. A. R. S. (2009). 7th International Symposium on Shiga Toxin (Verocytotoxin)-Producing Escherichia coli Infections (VTEC2009) Paper No. P03-2.2, p 71. Buenos Aires, Argentina. 2009.

Bosilevac, J. M., Nou, X., Osborn, M. S., Allen, D. M., and Koohmaraie, M. (2005a). Development and evaluation of an on-line hide decontamination procedure for use in a commercial beef processing plant. *J. Food Prot.* **68,** 265–272.

Bosilevac, J. M., Shackelford, S. D., Brichta, D. M., and Koohmaraie, M. (2005b). Efficacy of ozonated and electrolyzed oxidative waters to decontaminate hides of cattle before slaughter. *J. Food Prot.* **68,** 1393–1398.

Bosilevac, J., Wells, J., Arthur, T., Kalchayanand, N., Shackelford, S., Berry, E., Bono, J., Harhay, D., Wheeler, T., and Koohmaraie, M. (2009). *Escherichia coli* O157: H7 and *Salmonella* in Small Processing Plants and the Effect of Feeding Wet Distillers Grains with Solubles on *E. coli* O157: H7 USDA, NCBA Beef Industry Safety Summit, San Diego, CA.

Brandl, M. T. (2006). Fitness of human enteric pathogens on plants and implications for food safety. *Annu. Rev. Phytopathol.* **44,** 367–392.

Brandl, M. (2008). Plant lesions promote the rapid multiplication of *Escherichia coli* O157: H7 on postharvest lettuce. *Appl. Environ. Microbiol.* **74,** 5285.

Brandl, M., and Amundson, R. (2008). Leaf age as a risk factor in contamination of lettuce with *Escherichia coli* O157: H7 and *Salmonella enterica*. *Appl. Environ. Microbiol.* **74,** 2298–2305.

Brooks, J. T., Sowers, E. G., Wells, J. G., Greene, K. D., Griffin, P. M., Hoekstra, R. M., and Strockbine, N. A. (2005). Non-O157 Shiga toxin-producing *Escherichia coli* infections in the United States, 1983–2002. *J. Infect. Dis.* **192,** 1422–1427.

Brunder, W., Schmidt, H., and Karch, H. (1996). KatP, a novel catalase-peroxidase encoded by the large plasmid of enterohaemorrhagic *Escherichia coli* O157: H7. *Microbiology* **142,** 3305–3312.

Brussow, H. (2005). Phage therapy: The *Escherichia coli* experience. *Microbiology* **151,** 2133–2140.

Burt, S. (2004). Essential oils: Their antibacterial properties and potential applications in foods—A review. *Int. J. Food Microbiol.* **94,** 223–253.

Busz, H., McAllister, T., Yanke, L., Olson, M., Morck, D., and Read, R. (2002). Development of antibiotic resistance among *Escherichia coli* in feedlot cattle. *J. Anim. Sci.* **80,** 102.

Buzby, J. C. (2001). Children and microbial foodborne illness. *Food Rev.* **24,** 32–37.

Callaway, T., and Diez-Gonzalez, F. (2002). Pre-harvest intervention strategies to reduce *Escherichia coli* O157: H7 in cattle. *In* "Gastrointestinal Microbiology in Animals" (S. A. Martin, Ed.), pp. 19–40. Research Signpost, Kerala, India.

Callaway, T., Anderson, R., Genovese, K., Poole, T., Anderson, T., Byrd, J., Kubena, L., and Nisbet, D. (2002). Sodium chlorate supplementation reduces *E. coli* O157: H7 populations in cattle. *J. Anim. Sci.* **80,** 1683.

Callaway, T., Edrington, T., Rychlik, J., Genovese, K., Poole, T., Jung, Y., Bischoff, K., Anderson, R., and Nisbet, D. J. (2003). Ionophores: Their use as ruminant growth promotants and impact on food safety. *Curr. Issues Intest. Microbiol.* **4,** 43–51.

Callaway, T., Anderson, R., Edrington, T., Genovese, K., Bischoff, K., Poole, T., Jung, Y., Harvey, R., and Nisbet, D. (2004). What are we doing about *Escherichia coli* O157: H7 in cattle? *J. Anim. Sci.* **82,** E93.

Callaway, T., Anderson, R., Edrington, T., Genovese, K., Harvey, R., Poole, T., and Nisbet, D. (2007). Recent pre-harvest supplementation strategies to reduce carriage and shedding of zoonotic enteric bacterial pathogens in food animals. *Anim. Health Res. Rev.* **5,** 35–47.

Callaway, T. R., Edrington, T. S., Brabban, A. D., Anderson, R. C., Rossman, M. L., Engler, M. J., Carr, M. A., Genovese, K. J., Keen, J. E., and Looper, M. L. (2008). Bacteriophage isolated from feedlot cattle can reduce *Escherichia coli* O157: H7 populations in ruminant gastrointestinal tracts. *Foodborne Pathog. Dis.* **5,** 183–191.

Callaway, T. R., Carr, M., Edrington, T., Anderson, R. C., and Nisbet, D. J. (2009). Diet, *Escherichia coli* O157: H7, and cattle: A review after 10 years. *Curr. Issues Mol. Biol.* **11,** 67–79.

Calvin, L. (2007). Outbreak linked to spinach forces reassessment of Food Safety Practices. *Amber Waves* **5**(3), 24–31.

Castanie-Cornet, M. P., Penfound, T. A., Smith, D., Elliott, J. F., and Foster, J. W. (1999). Control of acid resistance in *Escherichia coli*. *J. Bacteriol.* **181,** 3525.

Casteel, M. J., Schmidt, C. E., and Sobsey, M. D. (2008). Chlorine disinfection of produce to inactivate hepatitis A virus and coliphage MS2. *Int. J. Food Microbiol.* **125,** 267–273.

Centers for Disease Control and Prevention (2006). Ongoing multistate outbreak of Escherichia coli serotype O157: H7 infections associated with consumption of fresh spinach—United States, September 2006. *Morb. Mortal. Wkly Rep.* **55,** 1045–1046.

Chang, S. T., Chen, P. F., and Chang, S. C. (2001). Antibacterial activity of leaf essential oils and their constituents from *Cinnamomum osmophloeum*. *J. Ethnopharmacol.* **77,** 123–127.

Charles, A. S., Baskaran, S. A., Murcott, C., Schreiber, D., Hoagland, T., and Venkitanarayanan, K. (2008). Reduction of *Escherichia coli* O157: H7 in Cattle drinking-water by trans-cinnamaldehyde. *Foodborne Pathog. Dis.* **5,** 763–771.

Cheville, A., Arnold, K., Buchrieser, C., Cheng, C., and Kaspar, C. (1996). *rpoS* regulation of acid, heat, and salt tolerance in *Escherichia coli* O157: H7. *Appl. Environ. Microbiol.* **62,** 1822–1824.

Chigor, V., Umoh, V., and Smith, S. (2010). Occurrence of *Escherichia coli* O157 in a river used for fresh produce irrigation in Nigeria. *Afr. J. Biotechnol.* **9,** 178–182.

Cobbold, R., Davis, M., Rice, D., Szymanski, M., Tarr, P., Besser, T., and Hancock, D. (2008). Association between bovine, human, and raw milk, and beef isolates of non-O157 Shiga toxingenic *Escherichia coli* within a restricted geographic area of the United States. *J. Food Prot.* **71,** 1023–1027.

Connerton, P., Loc Carrillo, C., Swift, C., Dillon, E., Scott, A., Rees, C., Dodd, C., Frost, J., and Connerton, I. (2004). Longitudinal study of *Campylobacter jejuni* bacteriophages and their hosts from broiler chickens. *Appl. Environ. Microbiol.* **70,** 3877–3883.

Cooley, M. B., Chao, D., and Mandrell, R. E. (2006). *Escherichia coli* O157: H7 survival and growth on lettuce is altered by the presence of epiphytic bacteria. *J. Food Prot.* **69,** 2329–2335.

Cooley, M., Carychao, D., Crawford-Miksza, L., Jay, M. T., Myers, C., Rose, C., Keys, C., Farrar, J., and Mandrell, R. E. (2007). Incidence and tracking of *Escherichia coli* O157: H7 in a major produce production region in California. *PLoS ONE* **2**(2), 1159.

Corbin, B. D., McLean, R. J., and Aron, G. M. (2001). Bacteriophage T4 multiplication in a glucose-limited *Escherichia coli* biofilm. *Can. J. Microbiol.* **47,** 680–684.

Dargatz, D. A., Wells, S. J., Thomas, L. A., Hancock, D. D., and Garber, L. P. (1997). Factors associated with the presence of *Escherichia coli* O157 in feces of feedlot cattle. *J. Food Prot.* **60,** 466–470.

Davey, M. E., and O'toole, G. A. (2000). Microbial biofilms: From ecology to molecular genetics. *Microbiol. Mol. Biol. Rev.* **64,** 847–867.

De Biase, D., Tramonti, A., Bossa, F., and Visca, P. (1999). The response to stationary-phase stress conditions in *Escherichia coli*: Role and regulation of the glutamic acid decarboxylase system. *Mol. Microbiol.* **32,** 1198–1211.

De Roever, C. (1998). Microbiological safety evaluations and recommendations on fresh produce. *Food Control* **9,** 321–347.

Delaquis, P., Bach, S., and Dinu, L. D. (2007). Behavior of *Escherichia coli* O157: H7 in leafy vegetables. *J. Food Prot.* **70,** 1966–1974.

DeVinney, R., Stein, M., Reinscheid, D., Abe, A., Ruschkowski, S., and Finlay, B. B. (1999). Enterohemorrhagic *Escherichia coli* O157: H7 produces Tir, which is translocated to the host cell membrane but is not tyrosine phosphorylated. *Infect. Immun.* **67,** 2389.

Dewell, G., Ransom, J., Dewell, R., McCurdy, K., Gardner, I., Hill, E., Sofos, J., Belk, K., Smith, G., and Salman, M. (2005). Prevalence of and risk factors for *Escherichia coli* O157 in market-ready beef cattle from 12 US feedlots. *Foodborne Pathog. Dis.* **2,** 70–76.

Di Pasqua, R., Hoskins, N., Betts, G., and Mauriello, G. (2006). Changes in membrane fatty acids composition of microbial cells induced by addiction of thymol, carvacrol, limonene, cinnamaldehyde, and eugenol in the growing media. *J. Agric. Food Chem.* **54,** 2745–2749.

Diez-Gonzalez, F., and Karaibrahimoglu, Y. (2004). Comparison of the glutamate-, arginine- and lysine-dependent acid resistance systems in *Escherichia coli* O157:H7. *J. Appl. Microbiol.* **96,** 1237–1244.

Duguid, J., Smith, I. W., Dempster, G., and Edmunds, P. (1955). Non-flagellar filamentous appendages (fimbriae) and haemagglutinating activity in *Bacterium coli*. *J. Pathol. Bacteriol.* **70,** 335–348.

Dykes, G., and Moorhead, S. (2002). Combined antimicrobial effect of nisin and a listeriophage against *Listeria monocytogenes* in broth but not in buffer or on raw beef. *Int. J. Food Microbiol.* **73,** 71–81.

Dziva, F., Vlisidou, I., Crepin, V. F., Wallis, T. S., Frankel, G., and Stevens, M. P. (2007). Vaccination of calves with EspA, a key colonisation factor of *Escherichia coli* O157: H7, induces antigen-specific humoral responses but does not confer protection against intestinal colonisation. *Vet. Microbiol.* **123,** 254–261.

Edrington, T., Callaway, T., Varey, P., Jung, Y., Bischoff, K., Elder, R., Anderson, R., Kutter, E., Brabban, A., and Nisbet, D. (2003). Effects of the antibiotic ionophores monensin, lasalocid, laidlomycin propionate and bambermycin on *Salmonella* and *E. coli* O157:H7 in vitro. *J. Appl. Microbiol.* **94,** 207–213.

el-Abagy, M., Dutka, B., Kamel, M., and el-Zanfaly, H. (1988). Incidence of coliphage in potable water supplies. *Appl. Environ. Microbiol.* **54,** 1632–1633.

Endo, Y., Mitsui, K., Motizuki, M., and Tsurugi, K. (1987). The mechanism of action of ricin and related toxic lectins on eukaryotic ribosomes. *J. Biol. Chem.* **262,** 5908–5912.

Endo, Y., Tsurugi, K., Yutsudo, T., Takeda, Y., Ogasawara, T., and Igarashi, K. (1988). Site of action of a Vero toxin (VT2) from *Escherichia coli* O157: H7 and of Shiga toxin on eukaryotic ribosomes. RNA N-glycosidase activity of the toxins. *FEBS J.* **171,** 45–51.

Erickson, M. C., and Doyle, M. P. (2007). Food as a vehicle for transmission of Shiga toxin producing *Escherichia coli*. *J. Food Prot.* **70,** 2426–2449.

Ethelberg, S., Olsen, K. E., Scheutz, F., Jensen, C., Schiellerup, P., Enberg, J., Petersen, A. M., Olesen, B., Gerner-Smidt, P., and Molbak, K. (2004). Virulence factors for hemolytic uremic syndrome, Denmark. *Emerg. Infect. Dis.* **10,** 842–847.

Fagan, P. K., Hornitzky, M. A., Bettelheim, K. A., and Djordjevic, S. P. (1999). Detection of Shiga-like toxin (stx1 and stx2), intimin (eaea), and enterohemorrhagic *Escherichia coli* (EHEC) hemolysin (EHEC hlyA) genes in animal feces by multiplex PCR. *Appl. Environ. Microbiol.* **65,** 868–872.

Farrah, S. R. (1987). Ecology of phage in freshwater environments. *In* "Phage ecology," S. M. Goyal, C. P. Gerba, and G. Bitton, (Eds.), pp. 125–136. John Wiley & Sons, Inc., New York.

Feng, P., Lampel, K. A., Karch, H., and Whittam, T. S. (1998). Phenotypic and genotypic changes in the emergence of *Escherichia coli* O157:H7. *J. Infect. Dis.* **177,** 1750–1753.

Fisher, I., and Meakins, S. (2006). Surveillance of enteric pathogens in Europe and beyond: Enter-net annual report for 2004. http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=3032, http://www.hpa.org.uk/hpa/inter/enter-net_reports.htm.

Food and Drug Administration. (2006). Questions and answers: Taco Bell *E. coli* O157:H7 lettuce outbreak, http://www.cfsan.fda.gov/dms/tacobqa.html.

Foster, J. W. (2000). Microbial responses to acid stress. *In* "Bacterial Stress Responses" G. Storz and R. Hengge-Aronis, (Eds.), pp. 99–115. ASM Press, Washington, DC.

Foster, J. W. (2004). *Escherichia coli* acid resistance: Tales of an amateur acidophile. *Nat. Rev. Microbiol.* **2,** 898–907.

Francis, G. A., Thomas, C., and O'beirne, D. (1999). The microbiological safety of minimally processed vegetables. *Int. J. Food Sci. Technol.* **34,** 1–22.

Franz, E., and van Bruggen, A. H. C. (2008). Ecology of *E. coli* O157: H7 and Salmonella enterica in the primary vegetable production chain. *Crit. Rev. Microbiol.* **34,** 143–161.

Franz, E., van Diepeningen, A. D., de Vos, O. J., and van Bruggen, A. H. C. (2005). Effects of cattle feeding regimen and soil management type on the fate of *Escherichia coli* O157: H7 and *Salmonella enterica* serovar Typhimurium in manure, manure-amended soil, and lettuce. *Appl. Environ. Microbiol.* **71,** 6165.

Franz, E., Semenov, A. V., Termorshuizen, A. J., de Vos, O., Bokhorst, J. G., and van Bruggen, A. (2008a). Manure-amended soil characteristics affecting the survival of *E. coli* O157: H7 in 36 Dutch soils. *Environ. Microbiol.* **10,** 313–327.

Franz, E., Semenov, A., and Van Bruggen, A. (2008b). Modelling the contamination of lettuce with *Escherichia coli* O 157: H7 from manure-amended soil and the effect of intervention strategies. *J. Appl. Microbiol.* **105,** 1569–1584.

Fremaux, B., Prigent-Combaret, C., and Vernozy-Rozand, C. (2008). Long-term survival of Shiga toxin-producing *Escherichia coli* in cattle effluents and environment: An updated review. *Vet. Microbiol.* **132,** 1–18.

Frenzen, P. D., Drake, A., and Angulo, F. J. (2005). Economic cost of illness due to *Escherichia coli* O157 infections in the United States. *J. Food Prot.* **68,** 2623–2630.
Friedman, M., Kozukue, N., and Harden, L. A. (2000). Cinnamaldehyde content in foods determined by gas chromatography—Mass spectrometry. *J. Agric. Food Chem.* **48,** 5702–5709.
Friedman, M., Henika, P., and Mandrell, R. (2002). Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella enterica*. *J. Food Prot.* **65,** 1545–1560.
Friesema, I., Sigmundsdottir, G., van der Zwaluw, K., Heuvelink, A., Schimmer, B., de Jager, C., Rump, B., Briem, H., Hardardottir, H., Atladottir, A., Gudmundsdottir, E., and van Pelt, W. (2008). An international outbreak of Shiga toxin-producing Escherichia coli O157 infection due to lettuce, September-October 2007. *Euro Surveill.* **13,** 19065.
Fron, M., Madeira, H., Richards, C., and Morrison, M. (1996). The impact of feeding condensed distillers byproducts on rumen microbiology and metabolism. *Anim. Feed Sci. Technol.* **61,** 1–4, 235–245.
Furuse, K. (1987). Distribution of coliphages in the environment: general considerations. *Phage Ecol.* 87–124.
Gale, E. F. (1946). The bacterial amino acid decarboxylases. *Adv. Enzymol.* **IV,** 1–31.
Garg, A. X., Suri, R. S., Barrowman, N., Rehman, F., Matsell, D., Rosas-Arellano, M. P., Salvadori, M., Haynes, R. B., and Clark, W. F. (2003). Long-term renal prognosis of diarrhea-associated hemolytic uremic syndrome: A systematic review, meta-analysis, and meta-regression. *J. Am. Med. Assoc.* **290,** 1360–1370.
Gasser, G., Gautier, C., Steck, A., Siebemann, R. E., and Oechslin, R. (1955). Hamolytisch-uremische Syndrome. Bilaterale Nierenindennekrosen bei akutenerworbenen hamolyticschem. *Anamein. Schweiz. Med. Woschenshr.* **85,** 905–909.
Gil, M. I., Selma, M. V., López-Gálvez, F., and Allende, A. (2009). Fresh-cut product sanitation and wash water disinfection: Problems and solutions. *Int. J. Food Microbiol.* **134,** 37–45.
Gomes, C., Da Silva, P., Moreira, R. G., Castell-Perez, E., Ellis, E. A., and Pendleton, M. (2009). Understanding *E. coli* internalization in lettuce leaves for optimization of irradiation treatment. *Int. J. Food Microbiol.* **135,** 238–247.
Griffin, P. M., and Tauxe, R. V. (1991). The epidemiology of infections caused by *Escherichia coli* O157: H7, other enterohemorrhagic *E. coli*, and the associated hemolytic uremic syndrome. *Epidemiol. Rev.* **13,** 60–85.
Guixa-Boixareu, N., Calderón-Paz, J., Heldal, M., Bratbak, G., and Pedrós-Alió, C. (1996). Viral lysis and bacterivory as prokaryotic loss factors along a salinity gradient. *Aquat. Microb. Ecol.* **11,** 215–227.
Gustafson, R. H. (1991). Use of antibiotics in livestock and human health concerns. *J. Dairy Sci.* **74,** 1428–1432.
Hedican, E. B., Medus, C., Besser, J. M., Juni, B. A., Koziol, B., Taylor, C., and Smith, K. E. (2009). Characteristics of O157 versus non-O157 Shiga toxin-producing *Escherichia coli* infections in Minnesota, 2000–2006. *Clin. Infect. Dis.* **49,** 358–364.
Helander, I. M., Alakomi, H. L., Latva-Kala, K., Mattila-Sandholm, T., Pol, I., Smid, E. J., Gorris, L. G. M., and von Wright, A. (1998). Characterization of the action of selected essential oil components on Gram-negative bacteria. *J. Agric. Food Chem.* **46,** 3590–3595.
Herold, S., Karch, H., and Schmidt, H. (2004). Shiga toxin-encoding bacteriophages–genomes in motion. *Int. J. Med. Microbiol.* **294,** 115–121.
Herriott, D. E., Hancock, D. D., Ebel, E. D., Carpenter, L. V., Rice, D. H., and Besser, T. E. (1998). Association of herd management factors with colonization of dairy cattle by Shiga toxin-positive *Escherichia coli* O157. *J. Food Prot.* **61,** 802–807.

Heuvelink, A. E., Zwartkruis-Nahuis, J., and de Boerl, E. (1997). Evaluation of media and test kits for the detection and isolation of *Escherichia coli* O157 from minced beef. *J. Food Prot.* **60,** 817–824.

Hilborn, E. D., Mermin, J. H., Mshar, P. A., Hadler, J. L., Voetsch, A., Wojtkunski, C., Swartz, M., Mshar, R., Lambert-Fair, M. A., and Farrar, J. A. (1999). A multistate outbreak of *Escherichia coli* O157: H7 infections associated with consumption of mesclun lettuce. *Arch. Intern. Med.* **159,** 1758–1764.

Hill, D. D., Owens, W. E., and Tchounwou, P. B. (2006). Prevalence of *Escherichia coli* O157: H7 bacterial infections associated with the use of animal wastes in Louisiana for the period 1996–2004. *Int. J. Environ. Res. Public Health* **3,** 107–113.

Houdt, R., Aertsen, A., Moons, P., Vanoirbeek, K., and Michiels, C. W. (2006). N-acyl-l-homoserine lactone signal interception by *Escherichia coli*. *FEMS Microbiol. Lett.* **256,** 83–89.

Hsu, S. Y. (2005). Effects of flow rate, temperature and salt concentration on chemical and physical properties of electrolyzed oxidizing water. *J. Food Eng.* **66,** 171–176.

Hu, T., Lin, Y., and Ho, C. (1985). Natural variation of chemical components of the leaf oil of *Cinnamomum osmophloeum* Kaneh. *Bull. Taiwan For. Res. Inst.* **78,** 18–25.

Huang, Y. R., Hung, Y. C., Hsu, S. Y., Huang, Y. W., and Hwang, D. F. (2008). Application of electrolyzed water in the food industry. *Food Control* **19,** 329–345.

Huff, W., Huff, G., Rath, N., Balog, J., Xie, H., Moore, P. Jr., and Donoghue, A. (2002). Prevention of *Escherichia coli* respiratory infection in broiler chickens with bacteriophage (SPR02). *Poult. Sci.* **81,** 437.

Huff, W., Huff, G., Rath, N., Balog, J., and Donoghue, A. (2003). Bacteriophage treatment of a severe *Escherichia coli* respiratory infection in broiler chickens. *Avian Dis.* **47,** 1399–1405.

Huff, W., Huff, G., Rath, N., Balog, J., and Donoghue, A. (2004). Therapeutic efficacy of bacteriophage and Baytril (enrofloxacin) individually and in combination to treat colibacillosis in broilers. *Poult. Sci.* **83,** 1944.

Hughes, K., Sutherland, I., and Jones, M. (1998). Biofilm susceptibility to bacteriophage attack: The role of phage-borne polysaccharide depolymerase. *Microbiology* **144,** 3039–3047.

Inward, C. D., Howie, A. J., Fitzpatrick, M. M., Rafaat, F., Milford, D. V., and Taylor, C. M. (1997). Renal histopathology in fatal cases of diarrhoea-associated haemolytic uraemic syndrome. *Pediatr. Nephrol.* **11,** 556–559.

Islam, M., Doyle, M. P., Phatak, S. C., Millner, P., and Jiang, X. (2004a). Persistence of enterohemorrhagic *Escherichia coli* O157: H7 in soil and on leaf lettuce and parsley grown in fields treated with contaminated manure composts or irrigation water. *J. Food Prot.* **67,** 1365–1370.

Islam, M., Morgan, J., Doyle, M. P., Phatak, S. C., Millner, P., and Jiang, X. (2004b). Persistence of *Salmonella enterica* serovar Typhimurium on lettuce and parsley and in soils on which they were grown in fields treated with contaminated manure composts or irrigation water. *Foodborne Pathog. Dis.* **1,** 27–35.

Jacob, M., Fox, J., Drouillard, J., Renter, D., and Nagaraja, T. (2008a). Effects of dried distillers' grain on fecal prevalence and growth of *Escherichia coli* O157 in batch culture fermentations from cattle. *Appl. Environ. Microbiol.* **74,** 38.

Jacob, M., Parsons, G., Shelor, M., Fox, J., Drouillard, J., Thomson, D., Renter, D., and Nagaraja, T. (2008b). Feeding supplemental dried distiller's grains increases faecal shedding of *Escherichia coli* O157 in experimentally inoculated calves. *Zoonoses Public Health* **55,** 125.

Jacob, M. E., Callaway, T. R., and Nagaraja, T. (2009a). Dietary interactions and interventions affecting *Escherichia coli* O157 colonization and shedding in cattle. *Foodborne Pathog. Dis.* **6,** 785–792.

Jacob, M. E., Fox, J. T., Drouillard, J. S., Renter, D. G., and Nagaraja, T. (2009b). Evaluation of feeding dried distiller's grains with solubles and dry-rolled corn on the fecal prevalence of *Escherichia coli* O157: H7 and *Salmonella* spp. in cattle. *Foodborne Pathog. Dis.* **6,** 145–153.

Janisiewicz, W., Conway, W., Brown, M., Sapers, G., Fratamico, P., and Buchanan, R. (1999). Fate of *Escherichia coli* O157: H7 on fresh-cut apple tissue and its potential for transmission by fruit flies. *Appl. Environ. Microbiol.* **65,** 1.

Jensen, E. C., Schrader, H. S., Rieland, B., Thompson, T. L., Lee, K. W., Nickerson, K. W., and Kokjohn, T. A. (1998). Prevalence of broad-host-range lytic bacteriophages of *Sphaerotilus natans, Escherichia coli,* and *Pseudomonas aeruginosa. Appl. Environ. Microbiol.* **64,** 575–580.

Joerger, R. (2003). Alternatives to antibiotics: Bacteriocins, antimicrobial peptides and bacteriophages. *Poult. Sci.* **82,** 640.

Johnson, E. A. (2003). Microbial adaptation and survival in foods. *In* "Microbial Stress Adaptation and Food Safety" A. E. Yousef and V. K. Juneja, (Eds.), pp. 75–103. CRC Press, Boca Raton, Florida.

Johnson, R., Durham, R., Johnson, S., MacDonald, L., Jeffrey, S., and Butman, B. (1995). Detection of *Escherichia coli* O157:H7 in meat by an enzyme-linked immunosorbent assay, EHEC-Tek. *Appl. Environ. Microbiol.* **61,** 386–388.

Johnson, R. P., Clarke, R. C., Wilson, J. B., Read, S. C., Rahn, K., Renwick, S. A., Sandhu, K. A., Alves, D., Karmali, M. A., and Lior, H. (1996). Growing concerns and recent outbreaks involving non-O157: H7 serotypes of verotoxigenic *Escherichia coli. J. Food Prot.* **59,** 1112–1122.

Johnson, K. E., Thorpe, C. M., and Sears, C. L. (2006). The emerging clinical importance of non-O157 Shiga toxin-producing *Escherichia coli. Clin. Infect. Dis.* **43,** 1587–1595.

Johnston, L. M., Jaykus, L. A., Moll, D., Anciso, J., Mora, B., and Moe, C. L. (2006). A field study of the microbiological quality of fresh produce of domestic and Mexican origin. *Int. J. Food Microbiol.* **112,** 83–95.

Johnston, M. A., Harrison, M. A., and Morrow, R. A. (2009). Microbial antagonists of *Escherichia coli* O157: H7 on fresh-cut lettuce and spinach. *J. Food Prot.* **72,** 1569–1575.

Juneja, V. K., and Friedman, M. (2008). Carvacrol and cinnamaldehyde facilitate thermal destruction of *Escherichia coli* O157: H7 in raw ground beef. *J. Food Prot.* **71,** 1604–1611.

Kaneko, K. E. N. I., Hayashidani, H., Ohtomo, Y., Kosuge, J., Kato, M., Takahashi, K., Shiraki, Y., and Ogawa, M. (1999). Bacterial contamination of ready-to-eat foods and fresh products in retail shops and food factories. *J. Food Prot.* **62,** 644–649.

Kaper, J. B. (2005). Pathogenic *Escherichia coli. Int. J. Med. Microbiol.* **295,** 355–356.

Kaper, J. B., and Karmali, M. A. (2008). The continuing evolution of a bacterial pathogen. *Proc. Natl. Acad. Sci. USA* **105,** 4535.

Kaper, J., Gansheroff, L., Wachtel, M., and O'Brien, A. (1998). Intimin-mediated adherence of Shiga toxin-producing *Escherichia coli* and attaching-and-effacing pathogens. *In* "*Escherichia coli* O157: H7 and Other Shiga Toxin-Producing *E. coli* Strains" J. B. Kaper and A. D. O'Brien, (Eds.), pp. 148–156. American Society for Microbiology, Washington, DC.

Karch, H., and Bielaszewska, M. (2001). Sorbitol-fermenting shiga toxin-producing Escherichia coli O157: H {−} strains: Epidemiology, Phenotypic and molecular characteristics, and microbiological diagnosis. *J. Clin. Microbiol.* **39,** 2043.

Karmali, M. A., Petric, M., Lim, C., Fleming, P. C., Arbus, G. S., and Lior, H. (1985). The association between idiopathic hemolytic uremic syndrome and infection by verotoxin-producing *Escherichia coli. J. Infect. Dis.* **151,** 775–782.

Keskinen, L. A., Burke, A., and Annous, B. A. (2009). Efficacy of chlorine, acidic electrolyzed water and aqueous chlorine dioxide solutions to decontaminate *Escherichia coli* O157: H7 from lettuce leaves. *Int. J. Food Microbiol.* **132,** 134–140.

Keusch, G. T., Grady, G. F., Mata, L. J., and McIver, J. (1972). The pathogenesis of Shigella Diarrhea: I. Enterotoxin production by *Shigella dysenteriae* 1. *J. Clin. Investig.* **51,** 1212–1217.

Kirk, M. D., McKay, I., Hall, G. V., Dalton, C. B., Stafford, R., Unicomb, L., Gregory, J., and Angulo, F. J. (2008). Foodborne disease in Australia: The OzFoodNet experience. *Clin. Infect. Dis.* **47,** 392–400.

Klopfenstein, T., Erickson, G., and Bremer, V. (2007). Board-invited review: Use of distillers byproducts in the beef cattle feeding industry. *J. Anim. Sci.* **86,** 1223–1231.

Komanapalli, I., and Lau, B. (1996). Ozone-induced damage of *Escherichia coli* K-12. *Appl. Microbiol. Biotechnol.* **46,** 610–614.

Konadu, E., Donohue-Rolfe, A., Calderwood, S. B., Pozsgay, V., Shiloach, J., Robbins, J. B., and Szu, S. C. (1999). Syntheses and immunologic properties of *Escherichia coli* O157 O-specific polysaccharide and Shiga toxin 1 B subunit conjugates in mice. *Infect. Immun.* **67,** 6191.

Konowalchuk, J., Speirs, J., and Stavric, S. (1977). Vero response to a cytotoxin of *Escherichia coli*. *Infect. Immun.* **18,** 775.

Koseki, S., Yoshida, K., Kamitani, Y., Isobe, S., and Itoh, K. (2004). Effect of mild heat pre-treatment with alkaline electrolyzed water on the efficacy of acidic electrolyzed water against *Escherichia coli* O157: H7 and Salmonella on lettuce. *Food Microbiol.* **21,** 559–566.

Kudva, I. T., Jelacic, S., Tarr, P. I., Youderian, P., and Hovde, C. J. (1999). Biocontrol of *Escherichia coli* O157 with O157-specific bacteriophages. *Appl. Environ. Microbiol.* **65,** 3767–3773.

Lange, R., and Hengge-Aronis, R. (1991). Identification of a central regulator of stationary-phase gene expression in *Escherichia coli*. *Mol. Microbiol.* **5,** 49–59.

Large, T. M., Walk, S. T., and Whittam, T. S. (2005). Variation in acid resistance among Shiga toxin-producing clones of pathogenic *Escherichia coli*. *Appl. Environ. Microbiol.* **71,** 2493–2500.

Law, D. (2000). Virulence factors of *Escherichia coli* O157 and other Shiga toxin-producing *E. coli*. *J. Appl. Microbiol.* **88,** 729–745.

Leffler, H., and Svanborg-Eden, C. (1981). Glycolipid receptors for uropathogenic *Escherichia coli* on human erythrocytes and uroepithelial cells. *Infect. Immun.* **34,** 920.

Leistner, L. (1992). Food preservation by combined methods. *Food Res. Int.* **25,** 151–158.

LeJeune, J., and Wetzel, A. (2007). Preharvest control of *Escherichia coli* O157 in cattle. *J. Anim. Sci.* **85,** E73.

LeJeune, J. T., Besser, T. E., and Hancock, D. D. (2001). Cattle water troughs as reservoirs of *Escherichia coli* O157. *Appl. Environ. Microbiol.* **67,** 3053.

Lema, M., Williams, L., and Rao, D. (2001). Reduction of fecal shedding of enterohemorrhagic *Escherichia coli* O157: H7 in lambs by feeding microbial feed supplement. *Small Rumin. Res.* **39,** 31–39.

Lenski, R. (1988). Dynamics of interactions between bacteria and virulent bacteriophage. *Adv. Microb. Ecol.* **10,** 1–44.

Leverentz, B., Conway, W. S., Alavidze, Z., Janisiewicz, W. J., Fuchs, Y., Camp, M. J., Chighladze, E., and Sulakvelidze, A. (2001). Examination of bacteriophage as a biocontrol method for *Salmonella* on fresh-cut fruit: A model study. *J. Food Prot.* **64,** 1116–1121.

Leverentz, B., Janisiewica, W., and Conway, W. S. (2003). Biological control of minimally processed fruits and vegetables. *In* "Microbial Safety of Minimally Processed Foods [M]" J. S. Novak, G. M. Sapers, and V. K. Juneja, (Eds.), pp. 319–332. CRC Press, Boca Raton, FL.

Li, H., Tajkarimi, M., and Osburn, B. I. (2008). Impact of vacuum cooling on *Escherichia coli* O157: H7 infiltration into lettuce tissue. *Appl. Environ. Microbiol.* **74,** 3138.

Lin, J., Lee, I. S., Frey, J., Slonczewski, J. L., and Foster, J. W. (1995). Comparative analysis of extreme acid survival in *Salmonella typhimurium, Shigella flexneri,* and *Escherichia coli*. *J. Bacteriol.* **177,** 4097–4104.

Lin, J., Smith, M., Chapin, K., Baik, H., Bennett, G., and Foster, J. (1996). Mechanisms of acid resistance in enterohemorrhagic *Escherichia coli*. *Appl. Environ. Microbiol.* **62,** 3094.

Loewen, P., Triggs, B., George, C., and Hrabarchuk, B. (1985). Genetic mapping of katG, a locus that affects synthesis of the bifunctional catalase-peroxidase hydroperoxidase I in *Escherichia coli*. *J. Bacteriol.* **162,** 661.

Loncarevic, S., Johannessen, G., and Rorvik, L. (2005). Bacteriological quality of organically grown leaf lettuce in Norway. *Lett. Appl. Microbiol.* **41,** 186–189.

Los, J. M., Golec, P., Wegrzyn, G., Wegrzyn, A., and Los, M. (2008). Simple method for plating *Escherichia coli* bacteriophages forming very small plaques or no plaques under standard conditions. *Appl. Environ. Microbiol.* **74,** 5113.

Lu, T. K., and Collins, J. J. (2007). Dispersing biofilms with engineered enzymatic bacteriophage. *Proc. Natl. Acad. Sci. USA* **104,** 11197.

Lynch, M., Tauxe, R., and Hedberg, C. (2009). The growing burden of foodborne outbreaks due to contaminated fresh produce: Risks and opportunities. *Epidemiol. Infect.* **137,** 307.

Ma, Z., Masuda, N., and Foster, J. W. (2004). GadE (YhiE) activates glutamate decarboxylase-dependent acid resistance in *Escherichia coli* K-12. *J. Bacteriol.* **186,** 7378–7389.

Mahmoud, B. S. M., Bachman, G., and Linton, R. H. (2009). Inactivation of *Escherichia coli* O157: H7, *Listeria monocytogenes*, *Salmonella enterica* and *Shigella flexneri* on spinach leaves by X-ray. *Food Microbiol.* **27,** 24–28.

Maki, D. G. (2006). Don't eat the spinach—Controlling foodborne infectious disease. *N. Engl. J. Med.* **355,** 1952.

Manning, S. D., Motiwala, A. S., Springman, A. C., Qi, W., Lacher, D. W., Ouellette, L. M., Mladonicky, J. M., Somsel, P., Rudrik, J. T., and Dietrich, S. E. (2008). Variation in virulence among clades of *Escherichia coli* O157: H7 associated with disease outbreaks. *Proc. Natl. Acad. Sci. USA* **105,** 4868.

March, S., and Ratnam, S. (1986). Sorbitol-MacConkey medium for detection of *Escherichia coli* O157: H7 associated with hemorrhagic colitis. *J. Clin. Microbiol.* **23,** 869–872.

Mates, A. K., Sayed, A. K., and Foster, J. W. (2007). Products of the *Escherichia coli* acid fitness island attenuate metabolite stress at extremely low pH and mediate a cell density-dependent acid resistance. *J. Bacteriol.* **189,** 2759–2768.

Matthews, L., Low, J., Gally, D., Pearce, M., Mellor, D., Heesterbeek, J., Chase-Topping, M., Naylor, S., Shaw, D., and Reid, S. (2006). Heterogeneous shedding of *Escherichia coli* O157 in cattle and its implications for control. *Proc. Natl. Acad. Sci. USA* **103,** 547.

McAllister, T., Bach, S., Stanford, K., and Callaway, T. (2006). Shedding of *Escherichia coli* O157: H7 by cattle fed diets containing monensin or tylosin. *J. Food Prot.* **69,** 2075–2083.

McNabb, S., Jajosky, R. A., Hall-Baker, P., Adams, D., Sharp, P., Worshams, C., Anderson, W., Javier, A., Jones, G., and Nitschke, D. (2008). Summary of notifiable diseases—United States, 2006. *Morb. Mortal. Wkly Rep.* **55,** 1–92.

Mead, P., Slutsker, L., Dietz, V., Mccaig, L., Breese, J., Shapiro, C., Griffin, P., Tauxe, R., Walker, D., and Dumler, J. (1999). Food-related illness and death in the United States. *Emerg. Infect. Dis.* **5,** 607–625.

Medellin-Pena, M. J., and Griffiths, M. W. (2009). Effect of molecules secreted by *Lactobacillus acidophilus* strain La-5 on *Escherichia coli* O157: H7 colonization. *Appl. Environ. Microbiol.* **75,** 1165–1171.

Meng, J., Doyle, M., Zhao, T., and Zhao, S. (2001). Enterohemorrhagic *Escherichia coli*. *In* "Food Microbiology: Fundamentals and Frontiers" M. P. Doyle, L. R. Beuchat, and T. J. Montville, (Eds.), 2nd edn., pp. 193–213. American Society for Microbiology, Washington, D.C.

Merrell, D. S., and Camilli, A. (2002). Acid tolerance of gastrointestinal pathogens. *Curr. Opin. Microbiol.* **5,** 51–55.

Michino, H., Araki, K., Minami, S., Takaya, S., Sakai, N., Miyazaki, M., Ono, A., and Yanagawa, H. (1999). Massive outbreak of *Escherichia coli* O157: H7 infection in schoolchildren in Sakai City, Japan, associated with consumption of white radish sprouts. *Am. J. Epidemiol.* **150,** 787.

Middelboe, M. (2000). Bacterial growth rate and marine virus–host dynamics. *Microb. Ecol.* **40,** 114–124.

Monday, S. R., Minnich, S. A., and Feng, P. C. H. (2004). A 12-base-pair deletion in the flagellar master control gene flhC causes nonmotility of the pathogenic German sorbitol-fermenting *Escherichia coli* O157: H-strains. *J. Bacteriol.* **186,** 2319.

Mukherjee, A., Speh, D., Dyck, E., and Diez-Gonzalez, F. (2004). Preharvest evaluation of coliforms, Escherichia coli, Salmonella, and *Escherichia coli* O157: H7 in Organic and conventional produce grown by Minnesota farmers. *J. Food Prot.* **67,** 894–900.

Mukherjee, A., Speh, D., Jones, A. T., Buesing, K. M., and Diez-Gonzalez, F. (2006). Longitudinal microbiological survey of fresh produce grown by farmers in the upper Midwest. *J. Food Prot.* **69,** 1928–1936.

Mukherjee, A., Speh, D., and Diez-Gonzalez, F. (2007). Association of farm management practices with risk of *Escherichia coli* contamination in pre-harvest produce grown in Minnesota and Wisconsin. *Int. J. Food Microbiol.* **120,** 296–302.

Nart, P., Naylor, S. W., Huntley, J. F., McKendrick, I. J., Gally, D. L., and Low, J. C. (2008). Responses of cattle to gastrointestinal colonization by *Escherichia coli* O157: H7. *Infect. Immun.* **76,** 5366.

Nataro, J. P., and Kaper, J. B. (1998). Diarrheagenic *Escherichia coli*. *Clin. Microbiol. Rev.* **11,** 142–201.

Naylor, S. W., Low, J. C., Besser, T. E., Mahajan, A., Gunn, G. J., Pearce, M. C., McKendrick, I. J., Smith, D. G. E., and Gally, D. L. (2003). Lymphoid follicle-dense mucosa at the terminal rectum is the principal site of colonization of enterohemorrhagic *Escherichia coli* O157: H7 in the bovine host. *Infect. Immun.* **71,** 1505.

Neetoo, H., Ye, M., and Chen, H. (2008). Potential application of high hydrostatic pressure to eliminate *Escherichia coli* O157: H7 on alfalfa sprouted seeds. *Int. J. Food Microbiol.* **128,** 348–353.

Nelson, S., LU, C., Beuchat, L., and Harrison, M. (2002). Radio-frequency heating of alfalfa seed for reducing human pathogens. *Trans. ASAE* **45,** 1937–1942.

Niemira, B. A. (2007). Relative efficacy of sodium hypochlorite wash versus irradiation to inactivate *Escherichia coli* O157: H7 internalized in leaves of Romaine lettuce and baby spinach. *J. Food Prot.* **70,** 2526–2532.

Niemira, B., Sommers, C., and Fan, X. (2002). Suspending lettuce type influences recoverability and radiation sensitivity of *Escherichia coli* O157: H7. *J. Food Prot.* **65,** 1388–1393.

Nishikawa, H., Yasuda, M., Uchiyama, J., Rashel, M., Maeda, Y., Takemura, I., Sugihara, S., Ujihara, T., Shimizu, Y., and Shuin, T. (2008). T-even-related bacteriophages as candidates for treatment of *Escherichia coli* urinary tract infections. *Arch. Virol.* **153,** 507–515.

Niu, Y., Johnson, R., Xu, Y., McAllister, T., Sharma, R., Louie, M., and Stanford, K. (2009). Host range and lytic capability of four bacteriophages against bovine and clinical human isolates of Shiga toxin-producing *Escherichia coli* O 157: H 7. *J. Appl. Microbiol.* **107,** 646–656.

Nowicki, B., Labigne, A., Moseley, S., Hull, R., Hull, S., and Moulds, J. (1990). The Dr hemagglutinin, afimbrial adhesins AFA-I and AFA-III, and F1845 fimbriae of uropathogenic and diarrhea-associated *Escherichia coli* belong to a family of hemagglutinins with Dr receptor recognition. *Infect. Immun.* **58,** 279.

O'Brien, A., and Holmes, R. (1987). Shiga and Shiga-like toxins. *Microbiol. Mol. Biol. Rev.* **51,** 206–220.

O'Brien, A., and LaVeck, G. (1983). Purification and characterization of a Shigella dysenteriae 1-like toxin produced by *Escherichia coli*. *Infect. Immun.* **40,** 675.

O'Flynn, G., Ross, R., Fitzgerald, G., and Coffey, A. (2004). Evaluation of a cocktail of three bacteriophages for biocontrol of *Escherichia coli* O157: H7. *Appl. Environ. Microbiol.* **70,** 3417.

Ogawa, M., Shimizu, K., Nomoto, K., Tanaka, R., Hamabata, T., Yamasaki, S., Takeda, T., and Takeda, Y. (2001). Inhibition of in vitro growth of Shiga toxin-producing *Escherichia coli* O157: H7 by probiotic *Lactobacillus* strains due to production of lactic acid. *Int. J. Food Microbiol.* **68,** 135–140.

Ogura, Y., Ooka, T., Iguchi, A., Toh, H., Asadulghani, M., Oshima, K., Kodama, T., Abe, H., Nakayama, K., and Kurokawa, K. (2009). Comparative genomics reveal the mechanism of the parallel evolution of O157 and non-O157 enterohemorrhagic *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **106,** 17939.

OIE. (2006). Manual of diagnostic tests and vaccine for terrestrial animals OIE, Paris, France, http://www.oie.int/manual-of-diagnostic-tests-and-vaccines-for-terrestrial-animals/.

Olsen, S., MacKinon, L., Goulding, J., Bean, N., and Slutsker, L. (2000). Surveillance for foodborne disease outbreaks—United States, 1993–1997. *Morb. Mortal. Wkly Rep. Surveill. Summ.* **49**(SS01), 1–62.

Oot, R., Raya, R., Callaway, T., Edrington, T., Kutter, E., and Brabban, A. (2007). Prevalence of *Escherichia coli* O157 and O157: H7-infecting bacteriophages in feedlot cattle feces. *Lett. Appl. Microbiol.* **45,** 445–453.

Ouattara, B., Simard, R. E., Holley, R. A., Piette, G. J. P., and Bégin, A. (1997). Antibacterial activity of selected fatty acids and essential oils against six meat spoilage organisms. *Int. J. Food Microbiol.* **37,** 155–162.

Pangloli, P., Hung, Y. C., Beuchat, L. R., King, C. H., and Zhao, Z. H. (2009). Reduction of *Escherichia coli* O157: H7 on produce by use of electrolyzed water under simulated food service operation conditions. *J. Food Prot.* **72,** 1854–1861.

Pao, S., Rolph, S., Westbrook, E., and Shen, H. (2004). Use of bacteriophages to control *Salmonella* in experimentally contaminated sprout seeds. *J. Food Sci.* **69,** 127–130.

Parkkinen, J., Ristimaki, A., and Westerlund, B. (1989). Binding of *Escherichia coli* S fimbriae to cultured human endothelial cells. *Infect. Immun.* **57,** 2256.

Paton, J. C., and Paton, A. W. (1998a). Pathogenesis and diagnosis of Shiga toxin-producing *Escherichia coli* infections. *Clin. Microbiol. Rev.* **11,** 450–479.

Paton, A. W., and Paton, J. C. (1998b). Detection and characterization of Shiga toxigenic *Escherichia coli* by using multiplex PCR assays for *stx1, stx2, eaeA*, enterohemorrhagic *E. coli* hlyA, rfbO111, and rfbO157. *J. Clin. Microbiol.* **36,** 598–602.

Penas, E., Gomez, R., Frías, J., and Vidal-Valverde, C. (2008). Application of high-pressure treatment on alfalfa (Medicago sativa) and mung bean (Vigna radiata) seeds to enhance the microbiological safety of their sprouts. *Food Control* **19,** 698–705.

Peterson, R., Klopfenstein, T., Moxley, R., Erickson, G., Hinkley, S., Bretschneider, G., Berberov, E., Rogan, D., and Smith, D. (2007). Effect of a vaccine product containing type III secreted proteins on the probability of *Escherichia coli* O157: H7 fecal shedding and mucosal colonization in feedlot cattle. *J. Food Prot.* **70,** 2568–2577.

Phillips, I. (1998). Antibiotic resistance from animals to man: Assessing the evidence. *J. Hosp. Infect.* **40,** H8F.

Potter, A. A., Klashinsky, S., Li, Y., Frey, E., Townsend, H., Rogan, D., Erickson, G., Hinkley, S., Klopfenstein, T., and Moxley, R. A. (2004). Decreased shedding of *Escherichia coli* O157: H7 by cattle following vaccination with type III secreted proteins. *Vaccine* **22,** 362–369.

Proctor, L. M., Okubo, A., and Fuhrman, J. A. (1993). Calibrating estimates of phage-induced mortality in marine bacteria: Ultrastructural studies of marine bacteriophage development from one-step growth experiments. *Microb. Ecol.* **25,** 161–182.

Puapermpoonsiri, U., Spencer, J., and van der Walle, C. (2009). A freeze-dried formulation of bacteriophage encapsulated in biodegradable microspheres. *Eur. J. Pharm. Biopharm.* **72,** 26–33.

Ramotar, K., Waldhart, B., Church, D., Szumski, R., and Louie, T. (1995). Direct detection of verotoxin-producing *Escherichia coli* in stool samples by PCR. *J. Clin. Microbiol.* **33,** 519–524.

Rasooly, R., and Do, P. M. (2010). Shiga toxin Stx2 is heat-stable and not inactivated by pasteurization. *Int. J. Food Microbiol.* **136,** 290–294.

Raya, R. R., Varey, P., Oot, R. A., Dyen, M. R., Callaway, T. R., Edrington, T. S., Kutter, E. M., and Brabban, A. D. (2006). Isolation and characterization of a new T-even bacteriophage, CEV1, and determination of its potential to reduce *Escherichia coli* O157: H7 levels in sheep. *Appl. Environ. Microbiol.* **72,** 6405–6410.

Reinstein, S., Fox, J., Shi, X., and Nagaraja, T. (2007). Prevalence of *Escherichia coli* O157: H7 in gallbladders of beef cattle. *Appl. Environ. Microbiol.* **73,** 1002–1008.

Riley, L., Remis, R., Helgerson, S., McGee, H., Wells, J., Davis, B., Hebert, R., Olcott, E., Johnson, L., and Hargrett, N. (1983). Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *N. Engl. J. Med.* **308,** 681–685.

Ronner, A., and Cliver, D. (1990). Isolation and characterization of a coliphage specific for *Escherichia coli* O157: H7. *J. Food Prot.* **53,** 944–947.

Rowbury, R., and Goodson, M. (1998). Induction of acid tolerance at neutral pH in log-phase *Escherichia coli* by medium filtrates from organisms grown at acidic pH. *Lett. Appl. Microbiol.* **26,** 447–451.

Roy, B., Ackermann, H., Pandian, S., Picard, G., and Goulet, J. (1993). Biological inactivation of adhering *Listeria monocytogenes* by listeriaphages and a quaternary ammonium compound. *Appl. Environ. Microbiol.* **59,** 2914.

Rozema, E. A., Stephens, T. P., Bach, S. J., Okine, E. K., Johnson, R. P., Stanford, K., and McAllister, T. A. (2009). Oral and rectal administration of bacteriophages for control of *Escherichia coli* O157: H7 in feedlot cattle. *J. Food Prot.* **72,** 241–250.

Sargeant, J., Sanderson, M., Smith, R., and Griffin, D. (2003). *Escherichia coli* O157 in feedlot cattle feces and water in four major feeder-cattle states in the USA. *Prev. Vet. Med.* **61,** 127–135.

Sargeant, J. M., Sanderson, M. W., Griffin, D. D., and Smith, R. A. (2004). Factors associated with the presence of *Escherichia coli* O157 in feedlot–cattle water and feed in the Midwestern USA. *Prev. Vet. Med.* **66,** 207–237.

Schmidt, H., Beutin, L., and Karch, H. (1995). Molecular analysis of the plasmid-encoded hemolysin of *Escherichia coli* O157: H7 strain EDL 933. *Infect. Immun.* **63,** 1055–1061.

Scott, L., McGee, P., Sheridan, J., Earley, B., and Leonard, N. (2006). A comparison of the survival in feces and water of *Escherichia coli* O157: H7 grown under laboratory conditions or obtained from cattle feces. *J. Food Prot.* **69,** 6–11.

Seymour, I., Burfoot, D., Smith, R., Cox, L., and Lockwood, A. (2002). Ultrasound decontamination of minimally processed fruits and vegetables. *Int. J. Food Sci. Technol.* **37,** 547–557.

Sharma, M., Ryu, J. H., and Beuchat, L. (2005). Inactivation of *Escherichia coli* O157: H7 in biofilm on stainless steel by treatment with an alkaline cleaner and a bacteriophage. *J. Appl. Microbiol.* **99,** 449–459.

Sharma, M., Patel, J. R., Conway, W. S., Ferguson, S., and Sulakvelidze, A. (2009). Effectiveness of bacteriophages in reducing *Escherichia coli* O157: H7 on fresh-cut cantaloupes and lettuce. *J. Food Prot.* **72,** 1481–1485.

Shaw, R. K., Berger, C. N., Feys, B., Knutton, S., Pallen, M. J., and Frankel, G. (2008). Enterohemorrhagic *Escherichia coli* exploit EspA filaments for attachment to salad leaves. *Appl. Environ. Microbiol.* **74,** 2908–2914.

Sheng, H., Knecht, H. J., Kudva, I. T., and Hovde, C. J. (2006). Application of bacteriophages to control intestinal *Escherichia coli* O157: H7 levels in ruminants. *Appl. Environ. Microbiol.* **72,** 5359–5366.

Shiga, K. (1898). Ueber den Dysenterie-bacillus (Bacillus dysenteriae). Zentralbl, Bakteriol. Orig.913.

Sivapalasingam, S., Friedman, C. R., Cohen, L., and Tauxe, R. V. (2004). Fresh produce: A growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. *J. Food Prot.* **67,** 2342–2353.

Small, P., Blankenhorn, D., Welty, D., Zinser, E., and Slonczewski, J. (1994). Acid and base resistance in *Escherichia coli* and *Shigella flexneri:* Role of *rpoS* and growth pH. *J. Bacteriol.* **176,** 1729.

Smith DeWaal, C., Barlow, K., Alderton, L., and Jacobson, M. (2002). Outbreak Alert Center for Science in the Public Interest, Washington, DC, www.cspinet.org/reports/outbreakpreport.pdf.

Smith, J., and Pillai, S. (2004). Irradiation and food safety. *Food Technol.* **58,** 48–55.

Smith, D. L., Harris, A. D., Johnson, J. A., Silbergeld, E. K., and Morris, J. G. Jr. (2002). Animal antibiotic use has an early but important impact on the emergence of antibiotic resistance in human commensal bacteria. *Proc. Natl. Acad. Sci. USA* **99,** 6434.

Smith, D. R., Moxley, R. A., Peterson, R. E., Klopfenstein, T. J., Erickson, G. E., and Clowser, S. L. (2008). A two-dose regimen of a vaccine against *Escherichia coli* O157: H7 type III secreted proteins reduced environmental transmission of the agent in a large-scale commercial beef feedlot clinical trial. *Foodborne Pathog. Dis.* **5,** 589–598.

Söderström, A., Lindberg, A., and Andersson, Y. (2005). EHEC O157 outbreak in Sweden from locally produced lettuce, August-September 2005. *Euro Surveill.* **10,** 1.

Söderström, A., Österberg, P., Lindqvist, A., Jönsson, B., Lindberg, A., Blide Ulander, S., Welinder-Olsson, C., Löfdahl, S., Kaijser, B., and De Jong, B. (2008). A large *Escherichia coli* O157 outbreak in Sweden associated with locally produced lettuce. *Foodborne Pathog. Dis.* **5,** 339–349.

Stanford, K., Croy, D., Bach, S., Wallins, G., Zahiroddini, H., and McAllister, T. (2005). Ecology of *Escherichia coli* O157: H7 in commercial dairies in southern Alberta. *J. Dairy Sci.* **88,** 4441.

Stewart, P. S., and William Costerton, J. (2001). Antibiotic resistance of bacteria in biofilms. *Lancet* **358,** 135–138.

Stone, R. (2002). Bactgeriophage therapy: Food and agriculture: Testing grounds for phage therapy. *Science* **298,** 730.

Sudarshana, M., Bandyopadhyay, S., Rosa, C., Suslow, T., and Harris, L. (2008). Effects of static and variable storage temperatures on the survival and growth of Escherichia coli O 157: H 7 on prewashed bagged lettuce. *Phytopathology* **98,** S152–S156.

Sulakvelidze, A., and Barrow, P. (2005). Phage therapy in animals and agribusiness. *In* "Bacteriophages: Biology and Applications" E. Kutter and A. Sulakvelidze, (Eds.), Bacteriophages: Biology and Applicationspp. 335–380. CRC Press, Boca Raton, FL.

Tait, K., Skillman, L., and Sutherland, I. (2002). The efficacy of bacteriophages as a method of biofilm eradication. *Biofouling* **18,** 305–311.

Takemasa, N., Ohnishi, S., Tsuji, M., Shikata, T., and Yokoigawa, K. (2009). Screening and analysis of spices with ability to suppress verocytotoxin production by *Escherichia coli* O157. *J. Food Sci.* **74,** 461–466.

Takeuchi, K., and Frank, J. F. (2000). Penetration of *Escherichia coli* O157: H7 into lettuce tissues as affected by inoculum size and temperature and the effect of chlorine treatment on cell viability. *J. Food Prot.* **63,** 434–440.

Talley, J., Wayadande, A., Wasala, L., Gerry, A., Fletcher, J., DeSilva, U., and Gilliland, S. (2009). Association of Escherichia coli O157: H7 with filth flies (Muscidae and Calliphoridae) captured in leafy greens fields and experimental transmission of E. coli O157: H7 to spinach leaves by house flies (Diptera: Muscidae). *J. Food Prot.* **174**(72), 1547–1552.

Tanji, Y., Shimada, T., Yoichi, M., Miyanaga, K., Hori, K., and Unno, H. (2004). Toward rational control of *Escherichia coli* O157: H7 by a phage cocktail. *Appl. Microbiol. Biotechnol.* **64,** 270–274.

Tarr, P. I. (2009). Shiga toxin-associated hemolytic uremic syndrome and thrombotic thrombocytopenic purpura: Distinct mechanisms of pathogenesis. *Kidney Int.* S29–S32.

Tarr, P. I., Gordon, C. A., and Chandler, W. L. (2005). Shiga-toxin-producing *Escherichia coli* and haemolytic uraemic syndrome. *Lancet* **365,** 1073–1086.

Tauxe, R. (1997). Emerging foodborne diseases: An evolving public health challenge. *Dairy Food Environ. Sanitation* **17,** 788–795.

Tauxe, R., Kruse, H., Hedberg, C., Potter, M., Madden, J., and Wachsmuth, K. (1997). Microbial hazards and emerging issues associated with produce: A preliminary report to the National Advisory Committee on Microbiologic Criteria for Foods. *J. Food Prot.* **60,** 1400–1408.

Team, C. F. E. R.California, Dept. of Health Services.Food and Drug Administration. (2007). Investigation of an *Escherichia coli* O157: H7 Outbreak Associated with Dole Pre-Packaged Spinach. California Department of Health Services, US Food and Drug Administration, San Francisco District.

Thomson, D. U., Loneragan, G. H., Thornton, A. B., Lechtenberg, K. F., Emery, D. A., Burkhardt, D. T., and Nagaraja, T. G. (2009). Use of a siderophore receptor and porin proteins-based vaccine to control the burden of *Escherichia coli* O157: H7 in feedlot cattle. *Foodborne Pathog. Dis.* **6,** 871–877.

Todd, E. C. D., Greig, J. D., Bartleson, C. A., and Michaels, B. S. (2009). Outbreaks where food workers have been implicated in the spread of foodborne disease. Part 6. Transmission and survival of pathogens in the food processing and preparation environment. *J. Food Prot.* **72,** 202–219.

Tyler, H. L., and Triplett, E. W. (2008). Plants as a habitat for beneficial and/or human pathogenic bacteria. *Annu. Rev. Phytopathol.* **46,** 53–73.

Uhlich, G., Sinclair, J., Warren, N., Chmielecki, W., and Fratamico, P. (2007). Characterization of Shiga toxin-producing Escherichia coli associated with two multi-state foodborne outbreaks that occurred in 2006. *Appl. Environ. Microbiol.* **74,** 1268–1272.

Unc, A., and Goss, M. J. (2004). Transport of bacteria from manure and protection of water resources. *Appl. Soil Ecol.* **25,** 1–18.

Vanaja, S. K., Springman, A. C., Besser, T. E., Whittam, T. S., and Manning, S. D. (2010). Differential expression of virulence and stress fitness genes between *Escherichia coli* O157: H7 strains with clinical or bovine-biased genotypes. *Appl. Environ. Microbiol.* **76,** 60–67.

VanBogelen, R., Kelley, P., and Neidhardt, F. (1987). Differential induction of heat shock, SOS, and oxidation stress regulons and accumulation of nucleotides in *Escherichia coli. J. Bacteriol.* **169,** 26.

Vicente, A., Civello, P., Martinez, G., Powell, A., Labavitch, J., and Chaves, A. (2005). Control of post-harvest spoilage in soft fruit. *Stewart Postharvest Rev.* **1,** 1–11.

Voetsch, A., Kennedy, M., Keene, W., Smith, K., Rabatsky-Ehr, T., Zansky, S., Thomas, S., Mohle-Boetani, J., Sparling, P., and McGavern, M. (2006). Risk factors for sporadic Shiga toxin-producing *Escherichia coli* O157 infections in FoodNet sites, 1999–2000. *Epidemiol. Infect.* **135,** 993–1000.

Vugia, D., Cronquist, A., Hadler, J., Tobin-D'Angelo, M., Blythe, D., Smith, K., Thornton, K., Morse, D., Cieslak, P., and Jones, T. (2006). Preliminary FoodNet data

on the incidence of infection with pathogens transmitted commonly through food—10 states, United States, 2005. *Morb. Mortal. Wkly Rep.* **55,** 392–395.

Weissinger, W., McWatters, K., and Beuchat, L. (2001). Evaluation of volatile chemical treatments for lethality to *Salmonella* on alfalfa seeds and sprouts. *J. Food Prot.* **64,** 442–450.

Welinder-Olsson, C., and Kaijser, B. (2005). Enterohemorrhagic *Escherichia coli* (EHEC). *Scand. J. Infect. Dis.* **37,** 405–416.

Welinder-Olsson, C., Stenqvist, K., Badenfors, M., Brandeberg, Å., Floren, K., Holm, M., Holmberg, L., Kjellin, E., Marild, S., and Studahl, A. (2004). EHEC outbreak among staff at a children's hospital—Use of PCR for verocytotoxin detection and PFGE for epidemiological investigation. *Epidemiol. Infect.* **132,** 43–49.

Wendakoon, C. N., and Sakaguchi, M. (1995). Inhibition of amino acid decarboxylase activity of *Enterobacter aerogenes* by active components in spices. *J. Food Prot.* **58,** 280–283.

White, D. (2000). How bacteria respond to environmental stress. *In* "The Physiology and Biochemistry of Prokaryotes," (D. White, Ed.), 2nd edn., pp. 534–548. Oxford University Press, New York.

Wick, L. M., Qi, W., Lacher, D. W., and Whittam, T. S. (2005). Evolution of genomic content in the stepwise emergence of *Escherichia coli* O157: H7. *J. Bacteriol.* **187,** 1783.

Williams, S., Mortimer, A., and Parry, J. (1999). Phages in soil. *In* "Encyclopedia of Virology" R. G. Webster and A. Eranoff, (Eds.), pp. 1248–1253. Academic Press, London.

Xavier, J. B., Picioreanu, C., Rani, S. A., van Loosdrecht, M. C. M., and Stewart, P. S. (2005). Biofilm-control strategies based on enzymic disruption of the extracellular polymeric substance matrix-a modelling study. *Microbiology* **151,** 3817–3832.

Yang, W., Ametaj, B., Benchaar, C., He, M., and Beauchemin, K. (2010). Cinnamaldehyde in feedlot cattle diets: Intake, growth performance, carcass characteristics, and blood metabolites. *J. Anim. Sci.* **88,** 1082.

Yavzori, M., Porath, N., Ochana, O., Dagan, R., Orni-Wasserlauf, R., and Cohen, D. (1998). Detection of enterotoxigenic *Escherichia coli* in stool specimens by polymerase chain reaction. *Diagn. Microbiol. Infect. Dis.* **31,** 503–509.

Ye, J., Kostrzynska, M., Dunfield, K., and Warriner, K. (2009). Evaluation of a biocontrol preparation consisting of *Enterobacter asburiae* JX1 and a lytic bacteriophage cocktail to suppress the growth of *Salmonella Javiana* associated with tomatoes. *J. Food Prot.* **72,** 2284–2292.

Ye, J., Kostrzynska, M., Dunfield, K., and Warriner, K. (2010). Control of *Salmonella* on sprouting mung bean and alfalfa seeds by using a biocontrol preparation based on antagonistic bacteria and lytic bacteriophages. *J. Food Prot.* **73,** 9–17.

Younts-Dahl, S. M., Osborn, G. D., Galyean, M. L., Rivera, J. D., Loneragan, G. H., and Brashears, M. M. (2005). Reduction of *Escherichia coli* O157 in finishing beef cattle by various doses of Lactobacillus acidophilus in direct-fed microbials. *J. Food Prot.* **68,** 6–10.

Zhou, B., Feng, H., and Luo, Y. (2009). Ultrasound enhanced sanitizer efficacy in reduction of Escherichia coli O157:H7 population on spinach leaves. *Journal of Food Science.* **74**(6), M308–M313.

CHAPTER TWO

Flue Gas Desulfurization Product Use on Agricultural Land

V. C. Baligar,* R. B. Clark,[†,1] R. F. Korcak,[‡,1] *and* R. J. Wright[‡,1]

Contents

* USDA-ARS, Beltsville Agricultural Research Center, Beltsville Maryland, USA
† USDA-ARS, Appalachian Farming Systems Research Center, Beaver, West Virginia, USA
‡ USDA-ARS, Beltsville, Maryland, USA
[1] Retired

Advances in Agronomy, Volume 111
ISSN 0065-2113, DOI: 10.1016/B978-0-12-387689-8.00005-9

Abstract

Combustion of coal produces over half of the electricity used in the United States and generates 123.5 m mt year^{-1} of coal combustion products (CCPs). Only about 45% of CCPs are beneficially utilized and the rest are discarded, mainly in landfills. One class of CCPs, called flue gas desulfurization (FGD) products, generated by removal of SO_2 from the exhaust gas of power plants, has physical and chemical properties that make them suitable for beneficial uses in agriculture. FGDs can be used as a soil amendment to provide a nutrient source for crops; ameliorate acidic soils; remediate sodic soils; improve soil structure to increase infiltration and water storage; reduce soil erosion and movement of sediments, nutrients, and pesticides to surface water; and stabilize and enrich organic composts and manures. FGD gypsum produced by a forced oxidation step following wet scrubbing of SO_2, is the most promising of the FGD materials for agricultural uses. FGD gypsum is comparable to commercially available mined gypsum. When applied to soil at agronomic rates, FGD gypsum appears to pose little environmental risk. However, more information is needed on risks associated with the introduction of trace elements such as Hg and As to the environment. Management practices for specific uses of FGDs also need to be developed across a range of soils, cropping systems, and climate regimes.

Abbreviations

ACAA	American Coal Ash Association
CCE	$CaCO_3$ equivalency
CCP(s)	coal combustion product(s)
CCT	clean coal technology
CWA	Clean Water Act
DM	dry matter
EC	electrical conductivity
FA(s)	fly ash(s)
FBC(s)	fluidized bed combustion product(s)
FGD(s)	flue gas desulfurization product(s)
m mt	million metric ton
TCLP	toxicity characteristic leaching procedure
USDA-ARS	U.S. Department of Agriculture, Agricultural Research Service
USEPA	U.S. Environmental Protection Agency

1. Introduction

Over half of the electricity used in the United States presently is produced by burning coal (D. Goss, 2008; ACAA, personal communication). When coal is burned for generation of electricity, considerable amounts of coal combustion products (CCPs) are produced (124 m mt in 2008) (ACAA, 1991–2009). Many of the CCPs could be used as mineral resources, but presently they are vastly underutilized. CCPs currently produced in the United States are underutilized compared to other developed countries. In addition, the amount of CCPs produced annually represents the third largest source of mineral resources in the United States. The amount of CCPs can be compared to other mineral resources: crushed stone, 1590 m mt; sand and gravel, 1170 m mt; iron ore, 52 m mt (U.S. Geological Survey, 2008) and Portland and masonry cement, 96 m mt (U.S. Geological Survey, 2007). Opportunities should be sought to utilize CCPs constructively and beneficially.

Over half of the CCPs in the United States presently are discarded, mainly in landfills. Landfill sites are becoming more limited and disposal costs continue to increase. The beneficial value of many CCPs is well established by research and commercial practices in the United States and elsewhere, and CCPs could be readily and beneficially used (Power and Dick, 2000). Otherwise, large amounts of the CCPs will have to be stored in landfills and/or at production sites in impoundment ponds or mountains of solids. Storage of large quantities of CCPs in a small area poses a significant environmental risk. While many of the barriers limiting beneficial use of CCPs have been addressed, overcoming these barriers has been slow (Pflughoeft-Hassett and Renninge, 1999).

Surveys conducted annually by ACAA on CCP production and use in the United States indicate that fly ash (FA) and flue gas desulfurization (FGD) production has increased over time and will likely continue to increase in the future (Table 1). In the ACAA surveys, CCPs are classified into FAs, bottom ashes, boiler slags, and FGDs based on their generation and characteristics. Percentage distribution by weight of CCPs in 2008 was: 53.3% FAs, 13.5% bottom ash, 1.5% boiler slag, and 24.8% FGDs (ACAA, 1991–2009). Although production of FGDs was not listed in the surveys until 1987, the volume of FGDs produced has increased steadily (Table 1) because new clean coal technology (CCT) systems have been or are being installed to meet legislative requirements of the Clean Air Act for reducing harmful emissions of sulfur oxides (SO_x) and nitrogen oxides (NO_x) into the atmosphere. In the 2008 ACAA CCP use survey about 42% of FAs, 44% of bottom ashes, 83% of boiler slags, and 60% of FGD gypsum (one of the FGD products) were being used beneficially (ACAA, 1991–2009).

Table 1 Annual production of FAs, FGDs, and total CCPs by coal burning power plants (1991–2008)[a]

Year	FAs (m mt year^{-1})	FGDs[b] (m mt year^{-1})	Total CCPs[c] (m mt year^{-1})
1991	46.6	16.4	80.6
1992	43.6	14.4	74.4
1993	43.4	18.5	80.4
1994	49.8	14.1	80.8
1995	49.2	18.1	83.7
1996	53.9	21.7	92.4
1997	54.7	22.8	95.4
1998	57.2	22.7	97.8
1999	56.9	22.3	97.2
2000	57.1	23.3	98.2
2001	61.8	25.8	107.1
2002	69.4	26.5	116.8
2003	63.6	28	110.5
2004	64.2	28.5	111.2
2005	64.5	28.2	111.8
2006	65.7	27.4	113.3
2007	65.1	30.1	114.7
2008	65.8	30.6	123.5
Increase (1991–2008)	19.2	14.2	42.9
% increase (1991–2008)	41.2	86.6	53.2

[a] Taken from ACAA (1991–2009).
[b] FGDs from 2002 to 2006 are comprised of FGD Gypsum, FGD Material Wet and Dry Scrubbers, and FGD Other.
[c] CCPs not listed are bottom ashes and boiler slags.

Beneficial use of FGDs could be on agricultural/pasture/forest land as an amendment to ameliorate acidic soils; reduce soil alkalinity problems (reduce Na saturation of sodic soils); improve tilth, water infiltration, and water storage in soils; provide a source of mineral nutrients (Ca, S, Mg, B) for plants; reduce erosion and movement of sediment, nutrients, and pesticides to surface water; and stabilize and enrich organic composts/manures and in special construction projects on agricultural land (e.g., animal containment feedlot pads, outside feed storage pads, and pond liners) (Clark *et al.*, 2001; DeSutter and Cihacek, 2009; USEPA, 2008c; Ritchey *et al.*, 1998a,b, 2000). Even though nonagricultural uses of CCPs such as cement/concrete/grout, road- sub-bases, flowable structural fills, mine-fill and mine-stability, waste stabilization/solidification and wallboard production

have been substantially greater than agricultural uses, application on agricultural land can be important in overall management of CCPs. Information about beneficial use of FGDs is limited because they are newer CCPs than FAs and bottom ashes, and less research has been conducted on FGDs. A recent review by Dick *et al.* (2006) extensively addresses benefits and barriers to land application of FGD products.

Present CCT systems for capturing (scrubbing) S involve reaction of Ca from limestone (some Mg may also be involved when dolomitic limestone is used) with S to form $CaSO_4/CaSO_3$ compounds. Electric utility production procedures normally include combusting/injecting of finely divided limestone ($CaCO_3$) and/or calcined lime [$CaO/Ca(OH)_2$] into systems or directly into flue gas streams to capture S as $CaSO_4/CaSO_3$. Materials formed from these processes are fluidized bed combustion (FBC) and/or FGD products, with FGDs being more prevalent with present-day technology. Some FBCs and FGDs contain unspent (unreacted) $CaCO_3$, CaO, and $Ca(OH)_2$ materials which provide considerable alkalizing properties.

The objectives of this communication are to: (i) provide background information about FGDs, (ii) provide research information about FGD use on agricultural land, and (iii) describe benefits, cautions, rates, and risks that might be important when these materials are used on land. This information should benefit industry, state and federal agencies, consultants, and producers who make decisions about agricultural uses of FGDs.[1]

2. Definition of FGD

Considerable confusion exists about the definition of FGDs. Even though FGD is an important coal combustion technology, the composition of end-products generated differ widely because of power plant design, conditions for burning, composition of coal, composition of limestone, processes for removal of contaminants, forced oxidation, and treatment of end-products (dewatering, mixing with other CCPs). Because of these vast differences, different materials are called FGDs. Many articles/communications have defined FGDs as mixtures of FA, $CaSO_4$, and unspent lime/limestone-based sorbents. A definition of the FGD process (Pflughoeft-Hassett *et al.*, 1999) is: "removal of the sulfur gases from the flue gases typically using a high-calcium sorbent such as lime or limestone. The three

[1] About the content of chapter: "Flue Gas Desulfurization (FGD) Product Use on Agricultural Land" is an overview of various aspects of FGD use. This chapter briefly discusses the chemical composition of FGDs and benefits of FGD addition to agricultural land. No attempt has been made to review all the available literature on all aspects of various FGD products, their chemical properties, and their use and effects on agricultural land. Readers are referred to the reference section of this chapter for additional reading to find excellent in-depth coverage of FGD.

primary types of FGD processes commonly used by utilities are wet scrubbers, dry scrubbers, and sorbent injection." A definition of FGD materials provided by the Coal Combustion By-product Information Network (1999) is: "The solid stabilized by-product material that results from the FGD scrubber system also referred to as scrubber sludge. It is produced when the stack gases are sent through pulverized limestone to remove sulfur dioxide. The resulting material consists of calcium sulfate/sulfite and is commonly combined with FA to dry and stabilize the material. Usually the material is thixotropic (a liquid or gel when stirred or shaken which becomes solid when allowed to stand) unless stabilized with FA or other dry materials. Some utilities produce an "oxidized" material which is primarily calcium sulfate or synthetic gypsum. The scrubber sludge may be dry, but is commonly delivered as filter cake. It is normally high in calcium sulfites and sulfates."

The material produced by the forced oxidation process generally is called FGD gypsum, however, other names including recaptured gypsum, by-product gypsum and synthetic gypsum also are used (USEPA, 2008c). The chemical makeup ($CaSO_4{\cdot}2H_2O$) of FGD gypsum and mined gypsum is the same, however, the amount and types of trace materials and unreacted sorbents found in FGD gypsum vary among power plants and coal sources (USEPA, 2008c). USEPA (2008c) states that over the next 10 years, annual production of FGD gypsum may double as existing power plants comply with the EPA's Clean Air Interstate Rule and other requirements. Recycling of FGDs can result in significant environmental benefits including reduced green house gas emission and, in addition, FGD gypsum may be less expensive than mined gypsum.

Much of the confusion about FGD products arises because other dry materials [e.g., FA, $CaCO_3$, CaO, $Ca(OH)_2$, or other product(s)] are added to or are included in the FGD material from dry scrubbers or FBC to make them acceptable as end-products for discard/use. Thus, many products called FGDs may not be FGD in the true sense, but are FGD plus some added material(s). These so-called FGDs react chemically and/or physically more like the properties of the added material(s) than the initial $CaSO_4$/$CaSO_3$ FGD product. Numerous examples appear in the literature of products being referred to as FGDs when the properties are those of the materials which have been added to FGDs. For example, $CaSO_4$ and $CaSO_3$ have different chemical properties from those of FAs, FBCs, $CaCO_3$, CaO, and $Ca(OH)_2$. More appropriate definitions for materials called FGDs might be Stabilized FGD (e.g., FGD + FA, FGD + $CaCO_3$, FGD + CaO/$Ca(OH)_2$, FGD + FBC, FGD + CaO/$Ca(OH)_2$ + $CaCO_3$), Oxidized FGD (e.g., FGD gypsum, high $CaSO_4$ FGD), or high $CaSO_3$ FGD. It is important that the material added to FGD be defined if the properties or reactions of such FGD products in the environment are to be understood.

3. Chemical Composition of FGDs and Associated Compounds

Chemical composition of FGD products vary depending on design of the power plant, operating conditions, and materials used as described previously. Chemical properties, element concentrations, and TCLP (toxicity characteristic leaching procedure, Method 1311 Fed. Reg., 1990) leachate values of several nonstabilized, and oxidized FGDs are provided in Tables 2 and 3. Even though nonstabilized FGDs will not normally be used on land because they are wet sludges and are difficult to handle and transport, information about these FGDs is provided to show differences in chemical properties compared with stabilized and oxidized FGDs, especially in regard to sulfite concentrations. Chemical composition of some FAs, FBCs, coal, and limestones (Table 4), and trace elements in nitrogen and phosphorus fertilizers, sewage sludges, and manures are provided (Table 5).

Stabilized FGDs [e.g., FGDs containing FA, FBCs, $CaCO_3$, CaO, $Ca(OH)_2$] generally have higher pH, calcium carbonate equivalent (CCE) values, and concentrations of mineral elements essential to plants compared with nonstabilized FGDs (original FGD material formed without receiving stabilizing materials) (Table 2). The major exception is lower S in stabilized compared to nonstabilized FGDs, which is expected since most stabilizing materials added to FGDs contain relatively low S (Clark *et al.*, 1995a). Nitrogen, the major mineral nutrient essential to plants, is not listed because N has relatively high volatility when coal is burned and is normally lost. Concentrations of trace elements of environmental concern (e.g., As, B, Ba, Cd, Cr, Cu, Hg, Mo, Ni, Pb, Se, Zn) generally are higher in stabilized than in nonstabilized FGDs (Table 2), and FAs, FBCs, and coal generally contains higher trace element concentrations than limestone, N and P fertilizers, sewage sludge, and manure (Tables 4 and 5). Oxidized FGDs (high $CaSO_4$ FGDs or FGD gypsum) generally have the lowest concentrations of most mineral elements compared to the nonstabilized and stabilized FGDs (high $CaSO_3$ FGDs) and many other CCPs or materials commonly applied on land (Tables 2, 4, and 5). TCLP leachate values (Table 3) for FGD gypsum are below maximum limits allowed in drinking water standards by USEPA (2008a) (Table 6). Concentrations of elements in CCPs and other materials vary extensively (Tables 2, 4, and 5), and information is needed for each material to determine if the material poses a risk to the environment or human health.

Recently Kosta *et al.* (2005) did an extensive chemical and physical properties evaluation of 59 dry FGD samples collected from 13 locations representing four major FGD scrubbing technologies. These FGDs are dominated by Ca, S, Al, Fe, and Si. Strong preferential partitioning into the acid insoluble residue (i.e., coal ash residue) was observed for Al, Ba, Be, Cr, Fe, Li, K, Pb, Si, and

Table 2 Chemical properties and element concentrations [means and ranges (in parentheses)] of nonstabilized, stabilized, and oxidized FGDs ($N = 3$ in each group)[a]

Property/element	Units	Nonstabilized	Stabilized	Oxidized
pH (FGD:water, 1:1)		8.6 (8.4–8.8)	10.0 (9.6–10.6)	9.0 (8.6–9.5)
pH (FGD:water, 2:1)		9.6 (9.4–9.8)	10.8 (10.4–11.3)	9.3 (9.0–9.6)
EC (FGD:water, 1:1)	dS m^{-1}	2.7 (2.2–3.5)	3.2 (2.1–4.7)	2.3 (1.7–3.4)
EC (FGD:water, 1:2)	dS m^{-1}	3.7 (3.4–4.0)	3.5 (2.8–4.6)	2.3 (1.7–3.3)
$CaCO_3$ equivalency (CCE)	%	36 (8–63)	48 (39–67)	6 (1–13)
Residue (after digestion)	%	10 (6–17)	35 (14–46)	6 (5–7)
Ca	g kg^{-1}	269 (220–299)	200 (163–272)	229 (209–240)
S	g kg^{-1}	205 (188–216)	127 (104–167)	204 (177–219)
SO_4	g kg^{-1}	49 (35–64)	18 (14–24)	203 (176–217)
SO_3	g kg^{-1}	156 (146–168)	109 (90–144)	1 (0.8–2.1)
Mg	g kg^{-1}	10.0 (8.8–11.9)	12.1 (6.5–22.8)	0.4 (0.2–0.5)[b]
K	g kg^{-1}	1.6 (0.6–2.6)	5.6 (4.4–6.5)	0.2 (<0.1–0.4)
Na	g kg^{-1}	0.9 (0.4–1.2)	1.2 (1.2–1.3)	0.6 (0.4–0.8)
Fe	g kg^{-1}	2.7 (1.0–5.3)	8.4 (1.7–14.9)	0.7 (0.4–1.0)
Al	g kg^{-1}	2.4 (0.9–4.1)	9.2 (5.5–12.3)	0.5 (<1.0–1.2)
Si	mg kg^{-1}	323 (156–448)	623 (585–643)	133 (43–306)
P	mg kg^{-1}	54 (4–79)	305 (29–660)	23 (<1–61)
Mn	mg kg^{-1}	94 (85–107)	197 (90–403)	113 (58–196)
B	mg kg^{-1}	81 (46–145)	148 (98–175)	<1 (<1)[c]
Zn	mg kg^{-1}	4.2 (2.4–6.1)	8.2 (2.5–14.3)	0.8 (<0.1–2.5)
Cu	mg kg^{-1}	0.7 (<0.1–2.1)	4.8 (<0.1–7.6)	<0.1 (<0.1–0.1)
Mo	mg kg^{-1}	2.1 (0.5–5.3)	6.1 (0.5–14.7)	0.8 (<0.1–1.6)
Co	mg kg^{-1}	1.8 (1.4–2.6)	4.1 (13–.6.3)	1.3 (0.4–2.1)
Ni	mg kg^{-1}	6.7 (4.6–9.5)	9.4 (1.5–17.3)	3.0 (0.7–6.1)
Ag	mg kg^{-1}	<0.01 (<0.01)	<0.01 (<0.01)	<0.01 (<0.01)

As	Data not provided since analytical method should be different			
Ba	mg k^{-1}	144 (106–174)	314 (244–353)	80 (75–85)
Be	mg kg^{-1}	106 (85–127)	80 (62–114)	93 (88–97)
Cd	mg kg^{-1}	<0.01 (<0.01)	<0.01 (<0.01)	0.05 (<0.01–0.14)
Cr	mg kg^{-1}	87 (72–104)	76 (65–91)	78 (74–86)
La	mg kg^{-1}	0.4 (<0.1–1.2)	4.6 (2.6–6.4)	2.2 (<0.1–5.5)
Li	mg kg^{-1}	7.6 (0.4–17.2)	45.4 (30.6–60.1)	0.5 (<0.1–1.6)
Pb	mg kg^{-1}	15 (8–28)	99 (3–218)	10 (2–17)
Sb	mg kg^{-1}	<0.07 (<0.07)	<0.07 (<0.07)	<0.07 (<0.07)
Sc	mg kg^{-1}	0.6 (<0.1–1.4)	2.8 (1.4–3.7)	0.2 (<0.1–0.3)
Se	Data not provided since analytical method should be different			
Sn	mg kg^{-1}	<0.08 (<0.08)	<0.08 (<0.08)	<0.08 (<0.08)
Sr	mg kg^{-1}	215 (205–232)	289 (217–397)	193 (175–230)
Ti	mg kg^{-1}	131 (70–199)	444 (300–526)	35 (<1–88)
Tl	mg kg^{-1}	<0.06 (<0.06)	2.2 (<0.1–6.4)	0.9 (<0.1–2.7)
V	mg kg^{-1}	6.2 (0.2–13.7)	30.7 (5.1–46.4)	3.3 (<0.1–9.5)

[a] Taken from Clark *et al.* (1995a).
[b] One oxidized FGD had added Mg [as $Mg(OH)_2$] at 22.7 g kg^{-1}.
[c] Excludes oxidized FGD with added Mg which had relatively high B (99 mg kg^{-1}).

Table 3 TCLP (toxicity characteristic leaching procedure) leachate values for nonstabilized, stabilized, and oxidized FGDs ($N = 3$ for each group, values in parentheses are ranges)[a]

Element	Nonstabilized ($mg\ L^{-1}$)	Stabilized ($mg\ L^{-1}$)	Oxidized ($mg\ L^{-1}$)
Ca	1250 (951–1480)	1637 (1440–1750)	966 (848–1120)
S	37.8 (36.6–38.9)	38.5 (27.5–52.6)	310.9 (36.1–824.0)
Mg	163 (133–200)	217 (152–360)	8 (4–12)[b]
K	21.8 (8.5–39.6)	22.2 (15.8–30.2)	35.2 (9.7–49.3)
Na	4.1 (2.5–5.7)	19.0 (10.0–35.3)	ND[c]
Fe	4.4 (0.5–7.3)	17.2 (0.4–33.0)	0.5 (0.2–1.0)
Si	3.1 (2.8–3.4)	7.1 (3.8–11.5)	9.0 (1.1–24.3)
P	0.1 (0.1)	0.2 (0.1–0.3)	0.4 (<0.1–0.7)
Mn	0.27 (0.21–0.32)	0.83 (0.54–1.00)	0.46 (<0.1–1.20)
B	3.5 (2.3–4.8)	7.8 (4.1–9.6)	10.5 (0.3–21.1)
Zn	0.10 (0.04–0.14)	0.26 (0.06–0.48)	0.10 (0.08–0.13)
Cu	0.07 (0.04–0.10)	0.08 (0.06–0.10)	0.14 (0.05–0.30)
Mo	0.18 (0.04–0.42)	0.14 (0.09–0.21)	0.05 (<0.03–0.10)
Co	0.10 (0.08–0.11)	0.13 (0.09–0.16)	0.16 (0.12–0.20)
As	<0.2 (<0.2)	<0.2 (<0.2)	0.3 (<0.2–0.5)
Ba	<0.01 (<0.01)	<0.01 (<0.01)	<0.01 (<0.01)
Cd	<0.02 (<0.02)	<0.02 (<0.02)	<0.02 (<0.02)
Cr	0.10 (0.06–0.15)	0.11 (0.09–0.13)	0.17 (0.06–0.40)
Hg	0.0007 (0.0002–0.0007)	0.0026 (0.0004–0.0043)	0.0002 (0.0002–0.0005)
Pb	<0.2 (<0.2)	<0.2 (<0.2)	<0.2 (<0.2)
Se	<0.2 (<0.2)	<0.2 (<0.2)	0.3 (<0.2–0.5)

[a] Taken from Clark *et al.* (1995a).
[b] One oxidized FGD had added Mg (22.7 $g\ kg^{-1}$) as $Mg(OH)_2$.
[c] ND, not determined.

V. Sulfur, Ca, and Mg occurred primarily in water or acid-soluble forms associated with the sorbents or scrubber reaction products.

4. Benefits of FGD Addition to Agricultural Land

4.1. Resource rather than waste

Recent contamination of soil and water by poorly stored FA has increased public concern about the safety of CCPs. The U.S. Environmental Protection Agency (EPA) proposed classifying CCPs as either hazardous waste or solid

Table 4 Element concentrations (ranges plus mean for FA_B) in various FAs, FBCs, coal, and limestones

Element	Units	FA_A[a]	FA_B[b]	FBC[c]	Coal[d]	Limestone[e]
Ca	$g\ kg^{-1}$	1.1–223	24.7 (4.4–137)	230–460	5.0–26.7	180–397
S	$g\ kg^{-1}$	0.4–64	3.6 (0.12–38.2)	30–171	3.8–53.2	< 0.1–13.5
N	$g\ kg^{-1}$	0.25–3.3	–	–	12	–
Mg	$g\ kg^{-1}$	0.4–77	5.4 (3.7–13.2)	3.6–14.0	1.0–2.5	0.4–129
K	$g\ kg^{-1}$	1.7–67	15.8 (5.6–21.9)	0.02–8.0	0.2–4.3	< 0.002
Na	$g\ kg^{-1}$	< 0.1–71	4.5 (1.0–20.2)	0.2–7.0	< 1–2	0.01–1.50
Fe	$g\ kg^{-1}$	1.0–276	88.2 (22.6–245)	< 1.0–16.0	3.2–43.2	0.1–31.1
Al	$g\ kg^{-1}$	1.0–208	131 (55–186)	1.6–20.0	4.3–30.4	0.1–21.5
Si	$g\ kg^{-1}$	0.7–318	235 (192–287)	346–675	5.8–60.9	0.3–154
P	$mg\ kg^{-1}$	1970–4850	1400 (436–3230)	68–500	–	10–3660
Mn	$mg\ kg^{-1}$	25–3000	200 (155–465)	125–685	6–181	20–3000
B	$mg\ kg^{-1}$	10–5000	–	8–171	1.2–356	< 1–21
Cl	$mg\ kg^{-1}$	13–1720	–	–	< 1–5600	–
Zn	$mg\ kg^{-1}$	14–3500	126 (11–235)	4–105	< 1–5600	< 1–1100
Cu	$mg\ kg^{-1}$	14–2200	104 (< 25–246)	< 1–19	1.8–185	< 0.3–125
Mo	$mg\ kg^{-1}$	1.2–236	–	0.12–13.4	< 1–73	< 0.1–92.3
Co	$mg\ kg^{-1}$	7.9–31.3	43 (< 0.05–< 100)	28–36	–	< 1–42
Ni	$mg\ kg^{-1}$	1.8–4300	149 (35–1040)	7–29	0.4–104	10–24
Ag	$mg\ kg^{-1}$	< 0.01–36	–	< 0.01- 10	0.04–0.08	5.2
As	$mg\ kg^{-1}$	< 0.06–6300	124 (28–301)	< 0.06–0.3	0.5–106	< 0.1–25
Ba	$mg\ kg^{-1}$	1–32,000	875 (76–3310)	40–388	150–250	0.1–25
Be	$mg\ kg^{-1}$	12–72	11 (< 0.05–26)	81–178	–	86–250
Br	$mg\ kg^{-1}$	–	–	–	–	0.04–0.1
Cd	$mg\ kg^{-1}$	< 0.1–130	11 (< 1–< 25)	< 0.01–0.5	< 1–6.5	39
Ce	$mg\ kg^{-1}$	–	–	–	–	12
Cr	$mg\ kg^{-1}$	3.6–900	145 (< 50–211)	9–154	< 1–610	10–15

(*Continued*)

Table 4 (*Continued*)

Element	Units	FA_A[a]	FA_B[b]	FBC[c]	Coal[d]	Limestone[e]
F	mg kg^{-1}	0.4–610	–		10–295	<10–1410
Hg	mg kg^{-1}	0.005–12	–	<0.05	0.01–1.6	0.05–<0.1
Pb	mg kg^{-1}	3–2120	93 (<1–134)	1.5–128	4–218	20–1250
Rb	mg kg^{-1}	–	–	–	–	3
Sb	mg kg^{-1}	<0.07–131	–	<0.07	0.2–14	15
Sc	mg kg^{-1}	6.7–21.0	–	0.9–2.9	–	1–16
Se	mg kg^{-1}	<0.1–134	9.0 (1.4–90.7)	<0.1–11.4	0.4–8	0.08–0.1
Sn	mg kg^{-1}	<0.08–22.0	–	<0.08	–	–
Sr	mg kg^{-1}	30–7600	–	116–312	100–150	75–610
Ti	mg kg^{-1}	857–6040	9070 (5020–13,280)	261–515	–	–
Tl	mg kg^{-1}	<0.06–0.4	–	<0.06–1.1	–	171

[a] From Bilski *et al.* (1995) and Clark *et al.* (1995a).
[b] From data provided by Ann Kim, Dep. Energy, Fed. Energy Tech. Center, Pittsburgh, PA (number of samples comprising the mean = 27).
[c] From Alcordo and Rechcigl (1995), Clark *et al.* (1995a), Page *et al.* (1979), and Stout *et al.* (1988).
[d] From Bilski *et al.* (1995).
[e] From Alloway (1990), Chichilo and Whittaker (1961), Kabata-Pendias and Pendias (1992), and R. B. Clark, unpublished data.

Table 5 Trace element concentration ranges in nitrogen and phosphorus fertilizers, sewage sludge, and manure

Element	Nitrogen fertilizer[a] (mg kg^{-1})	Phosphorus fertilizer[a] (mg kg^{-1})	Sewage sludge[a] (mg kg^{-1})	Manure[b] (mg kg^{-1})
As	2.2–120	2–1200	2–30	3–25
B	–	5–115	15–1000	0.3–0.6
Ba	–	–	150–4000	270
Be	185–716	200	4–13	–
Br	–	3–5	20–65	16–41
Cd	0.05–8.5	0.1–170	$<$1–3410	0.3–0.8
Ce	–	20	20	–
Co	5.4–12	1–12	1–260	0.3–24
Cr	–	66–245	8–40,600	5.2–55
Cu	$<$1–15	1–300	50–8000	2–60
F	3.2–19	8500–38,000	2–740	7
Ge	–	–	1–10	19
Hg	0.3–2.9	0.01–1.2	0.1–55	0.09–0.2
In	–	–	–	1.4
Mn	–	40–2000	60–3900	30–350
Mo	1–7	–	1–40	0.05–3
Ni	7–34	7–38	6–5300	7.8–30
Pb	2–27	7–225	29–3600	6.6–15
Rb	–	5	4–95	0.06
Sc	–	7–36	0.5–7	5
Sb	–	–	3–44	–
Se	–	0.5–25	1–10	2.4
Sr	–	25–500	40–360	80
Sn	1.4–16.0	3–19	40–700	3.8
Te	–	20–23	–	–
U	–	30–300	–	–
V	–	2–1600	20–400	–
Zn	1–42	50–1450	91–49,000	–
Zr	–	–	5–90	–

[a] Modified from Alloway (1990) and Kabata-Pendias and Pendias (1992).
[b] Modified from Kabata-Pendias and Pendias (1992).

waste. Many FGD materials, especially FGD gypsum, present relatively little risk to the environment and would be classified as solid waste that potentially could be used in agriculture. Attempts have been made to remove some of the regulatory barriers to beneficial use of FGDs. Several states have approved agricultural uses of FGD gypsum and USEPA has promoted beneficial uses of FGD materials through the Coal Combustion Products Partnership.

Table 6 Standard limits for trace elements in drinking water, TCLP leachates, and CWA-503 waste regulations

Element	Drinking water[a] (mg L^{-1})	TCLP Leachate[b] (mg L^{-1})	Final CWA-503 limits[c]	
			Ceiling (mg kg^{-1})	Cumulative (kg ha^{-1})
Ag	–	5	–	–
As	0.01	5	75	41
Ba	2	100	–	–
Be	0.004	–	–	–
Cd	0.005	1	85	39
Cr	0.1	5	3000	3000
Cu	1.3	–	4300	1500
Hg	0.002	0.2	57	17
Mo	–	–	75	–
Ni	–	–	420	420
Pb	0.001	5	840	300
Se	0.05	1	100	100
Zn	–	–	7500	2800

[a] Values taken from USEPA (2008a).
[b] Values taken from USEPA (2008b).
[c] Values taken from USEPA (1994).

An appropriate beneficial use of FGDs would be application to agricultural land to ameliorate soil chemical and physical limitations, reduce soil erosion, protect water quality, promote more efficient soil capture of rainfall and improve crop production (Power and Dick, 2000). Soils are effective in buffering and/or diluting harmful effects that might result when amendments are added to soils. This could be important for management of FGDs, which are among the most benign of underutilized CCT products. Even so, sufficient information must be obtained and made available to eliminate hazards, overcome perceived barriers, promote safe use, and provide defined benefits for the various FGDs.

4.2. Mitigation of soil acidity

One of the beneficial uses of FGDs on land could be as an amendment to ameliorate acidic soils. Some FGDs are alkaline and can serve as liming agents. FGD gypsum is not a liming agent but it can help ameliorate acidic soils. FGD gypsum is particularly useful on many acidic soils that have sufficiently low pH (pH <5) and Ca status to induce detrimental and toxic effects to plants (Foy, 1992; Sumner *et al.*, 1991). Low pH and low

calcium status lead to greater availability of elements such as Al and Mn, which are toxic to root growth if present at sufficiently high levels (Foy, 1992), lower availability of mineral nutrients essential to plants, especially P (Mengel and Kirkby, 1982), and greater availability of trace elements such as Cd, Cr, Pb, and Ni, which may cause detrimental effects to plants as well as animals or humans when sufficient quantities of plant materials containing these elements are consumed (Kabata-Pendias and Adriano, 1995).

Many detrimental effects in acidic soils can be alleviated by increasing pH (>5.0–5.5). Although calcitic ($CaCO_3$) and dolomitic ($CaCO_3$/$MgCO_3$) limestones have been common amendments used worldwide to increase soil pH, many of the FGDs also increase soil pH when they contain alkalizing agent(s) [CaO/MgO, $Ca(OH)_2$/$Mg(OH)_2$, $CaCO_3$/$MgCO_3$]. One major problem with limestone is that the main constituent ($CaCO_3$) is relatively insoluble and generally is effective only at the site of placement or incorporation in soil (usually surface layer) and is not readily leached to subsurface soil layers (Ritchey *et al.*, 1995a, 1997). Thus, soils often must be disturbed or cultivated to distribute limestone in the profile so that it comes in contact with greater volumes of soil or with soil in deeper profiles. Tilling or disturbing soil is common practice for production of row crops (corn, soybeans), but not for pasture, perennial, and shrub or tree plants. However, often it is not possible or economically and environmentally feasible to use tillage to introduce limestone or alkaline FGDs into the soil profile. FGD gypsum is considerably more soluble than $CaCO_3$ (Korcak, 1998b), and can leach from surface to deeper soil layers (Farina *et al.*, 2000a,b; Dick *et al.*, 2000; Ritchey *et al.*, 1995a, 2000; Toma *et al.*, 1999). Enhanced concentrations of Ca and S (as SO_4) leached into subsoil layers can provide roots with these needed mineral nutrients, reduce availability of some toxic elements (Al, Fe, Cd, Cr, Pb), increase availability of other mineral nutrients (P, Zn, Cu, Mo), and promote root growth deeper into the soil profile. This increases crop tolerance to drought. Surface application of FGD gypsum has the potential to provide these benefits without disturbance of soil. In a West Virginia acidic Gilpin silt loam soil dissolution of applied FGD gypsum significantly increased exchangeable Ca and Mg and decreased exchangeable Al in the upper 0–15 cm soil profile resulting in increased soil pH (Zhou *et al.*, 2006). In this study, mobility of Ca and Mg through the soil profile was due to the presence of S in the FGD.

4.3. Source of nutrients to plants

FGD gypsum is an excellent source of Ca and S. FGD gypsum application can improve yield and quality of high Ca requiring plants such as peanuts, tomatoes and cantaloupes (USEPA, 2008c). Sulfur fertilization is required for many crops and FGD gypsum can be an effective source of S (USEPA, 2008c). FGD gypsum improved alfalfa and soybean yields and did not

impose environmental risks when applied to agricultural soils (Chen *et al.*, 2005). Application of FGD gypsum (33 kg S ha^{-1}) with N (0–233 kg N ha^{-1}) promoted corn growth and uptake of N in a silt loam soil of Ohio (Chen *et al.*, 2008). Other nutrients were supplied by FAs and/or other stabilizing materials added to FGDs. FGDs have been used as a source of B for soybean (Ransome and Dowdy, 1987). Selenium, which is required by many animals and plants (McDowell, 1992; Miller *et al.*, 1991), can be supplied by FGD amendments.

4.4. Improvement of soil physical properties

FGD application to land can improve soil physical properties. Soils with added FGDs have less surface crusting and compaction, greater water infiltration and water holding capacity, greater aggregate stability, and less water runoff and erosion (Korcak, 1998b; Norton and Zhang, 1998). Surface soil crusting often is prevented during rainfall events if FGDs are applied to enhance water penetration into soil and reduce erosion. The FGDs provide electrolytes to overcome dispersion of soil particles. Of the major electrolytic elements (Al, Ca, Mg, K, Na), Ca has the greatest ability to enhance flocculation (aggregation) of soil particles, particularly clay, to keep soils more friable, enhance water penetration, and improve root penetration into hard/compact soil layers (Norton and Zhang, 1998).

4.5. Reduction of runoff and soil erosion

Soil-applied FGD gypsum releases electrolytes that prevent soil surface sealing, thereby preventing a leading cause of soil erosion. The Ca in FGD gypsum reduces dispersion of soil particles by promoting flocculation and aggregation of clay particles (Ritchey, *et al.*, 2000). FGD gypsum applied to soil improves soil aggregation and rainfall infiltration and decreases runoff and soil erosion (USEPA, 2008c). Reduced crusting and improved aggregation due to applied FGD gypsum allows for greater water infiltration and soil water holding capacity. More effective soil storage and use of rainfall can help address crop water requirements during droughts. Research conducted in Indiana and Mississippi showed that application of 3 tons $acre^{-1}$ of FGD gypsum significantly reduces soil loss and runoff during natural rainfall events (Norton, 1995; Norton *et al.*, 1998). These results show that FGD gypsum can effectively control soil loss by erosion. Additional research will be needed in various ecosystems with a range of crops, soils, and management systems to fully document the effectiveness, safety, and environmental benefits of FGD gypsum for erosion control.

4.6. Mitigation of sodic soil

Application of $CaSO_4$ products, especially gypsum, can alleviate soil compaction (dispersion of soil particles) caused by high Na saturation and thereby improve water penetration in sodic soils (Levy, 2000; Ritchey *et al.*, 2000). Calcium replaces Na on clay exchange sites to enhance soil flocculation and stability which promotes aggregation of clay particles leading to better soil structure and stability and preventing soil crusting (USEPA, 2008c; Levy, 2000; Norton and Zhang, 1998). However, caution is needed when using FGDs that contain relatively high Na because Na promotes dispersion of clay particles and reduces water infiltration in soil. Chun *et al.* (2007) reported that application of 23.1 Mg ha^{-1} of FGD in salt-affected soils of northern China significantly increased EC, exchangeable and soluble Ca^{2+}, and $(SO_4)^{2-}$ and decreased $(CO_3)^{2-}$ and exchangeable and soluble Na^+. If FGD gypsum is used, information about gypsum use on land is applicable. Several excellent articles about gypsum use on land are available (Alcordo and Rechcigl, 1993, 1995; USEPA, 2008c; Korcak, 1998b; Levy, 2000; Miller, 1995; Ritchey *et al.*, 1995a, 1997, 2000; Shainberg *et al.*, 1989). Wang *et al.* (2008) demonstrated alkali soil amelioration by FGD gypsum application. FGD application (20.9–59.3 Mg ha^{-1}) improved corn (grain/forage) and alfalfa yield on alkaline soil.

4.7. Reduction of phosphorus availability/transport

Another benefit of FGD use on land is reduced movement of P from high-P soils where large amounts of P-containing materials (e.g., poultry manure) have been applied to land. Some major cropping areas of the United States contain higher levels of P than are recommended for agricultural crop production (Sharpley *et al.*, 1994). High levels of P in surface soil can lead to P loss in runoff water and subsequent pollution of surface waters. For example, outbreaks of the toxic dinaflagellate alga *Pfiesteria piscidia* in waterways of the eastern United States have been attributed to high levels of P in runoff water (Sharpley *et al.*, 1999). Application of FGDs with high $CaSO_4$ content convert P in soil to less soluble forms, which reduces runoff and transport of P to surface waters (Stout *et al.*, 1998; 2000), and potentially reduces P losses through leaching (USEPA, 2008c; He *et al.*, 1996a,b). Excess P in runoff leads to water quality problems, including algal blooms and eutrophication of water bodies (USEPA, 2008c). Surface drainage ditches on the Delmarva Peninsula transport P to sensitive bodies of water such as the Chesapeake Bay. FGD gypsum-filled trenches removed 50–95% of soluble P that was carried by lateral groundwater flow to surface drainage ditches which flow into the Chesapeake Bay (Bryant *et al.*, 2010).

4.8. Miscellaneous benefits

FGDs mixed with stabilization materials have been used successfully to create pads to keep animals on solid surfaces and dry during wet seasons of the year (Butalia *et al.*, 1999; Korcak, 1998a). Solid pads for storage and preservation of dried hay for feeding animals during the winter season also have been constructed (Butalia *et al.*, 1999). FGD materials (Butalia *et al.*, 1999; Wolfe *et al.*, 1999) have been used to make impermeable liners for water storage ponds.

Another beneficial use of FGDs has been to combine them with one or more other by-products. FGDs, as well as other CCPs, have been successfully combined with various organic materials (e.g., animal manures, biosolids, yard wastes, municipal solid wastes) for land and landscape application (Brown *et al.*, 1998; Power and Dick, 2000; Ritchey *et al.*, 1998b). The FGDs provide plant nutrients like Ca, S, and B, while organic matter provides nutrients like N and P. Organic matter also is important in maintaining/improving soil structure, water holding capacity, detoxification of Al by chelation and soil quality. FGDs with high alkalinity have been used as sterilizing and enrichment agents in composting of many organic materials (e.g., biosolids, yard/wood/industrial wastes, manures) (Logan and Burnham, 1995) and to kill detrimental pathogens while enriching the final product with minerals.

5. Cautions for FGD Use on Agricultural Land

5.1. Soil pH

FGD gypsum even at high application rates does not increase the pH of acidic soil. Increases in soil pH occur when alkalinizing agents are present in the FGDs. Stabilized FGDs can increase soil pH of acidic soil considerably, sometimes to undesirably high values, even at relatively low application rates (Clark *et al.*, 1995a). For example, FBC containing CaO products increased soil pH to >9, and detrimentally affected corn growth. Soil pH >8 is normally detrimental to growth of many plants. Optimal soil pH for growth of specific plant species varies, and may be related to reduced availability of toxic elements (Al, Mn) and increased availability of essential mineral nutrients. Reduced availability of essential mineral nutrients like Fe, Mn, Zn, and Cu usually occurs when soil pH exceeds 7 (Chen and Barak, 1982; Graham *et al.*, 1988; Marschner, 1995; Robson, 1993). Basing FGD application rates on soil lime requirements and CCE values of FGDs can reduce the risk of increasing soil pH to excessive values.

5.2. Excessive soluble salts

Stabilized FGDs normally contain many soluble salts. These can be detrimental to plants grown in soils amended with FGDs if application rates are excessive. This is especially true when excess B is added. Plants vary in sensitivity to soluble salts and, if inappropriate levels are present, seed germination, plant establishment, and growth may be reduced. Plants considered sensitive or moderately sensitive to salt normally tolerate EC values near 1.5 and 3.5 dS m^{-1}, respectively, before detrimental effects occur (Maas, 1990). Detrimental soluble salt effects would not be expected from application of most FGDs unless large amount are applied, and the risk from adding high levels of soluble salts is normally not a problem in the absence of nutrient imbalances and/or excessive B. If application and planting occur simultaneously, or very near each other, detrimental seed germination/seedling growth effects may occur because of concentrated salt levels in a specific area. FGDs weathered in open spaces where rain interacts with the materials generally have low soluble salts because of leaching.

5.3. Calcium imbalances with other mineral nutrients

FGDs contain high amounts of Ca which may cause imbalances of other mineral nutrients such as Mg, K, and P and induce deficiencies of these minerals in plants (Korcak, 1998a), especially when added to acidic soil (Clark *et al.*, 1997b; Punshon *et al.*, 1999). For example, Mg deficiency was common when corn was grown with various FGDs added at different levels (R. B. Clark, USDA-ARS, Beaver, WV, personal observations). Once Mg was added to provide soil Ca:Mg ratios of approximately 30:1 were achieved, Mg deficiency symptoms were alleviated (Clark *et al.*, 1997b). Differences in effectiveness among various sources of Mg for enhancing plant growth and alleviating Mg deficiency also were observed (Zeto *et al.*, 1997). A FGD product developed to alleviate Mg deficiency associated with FGD gypsum use (College *et al.*, 1997), enhanced growth of corn and several forage plant species when applied at low rates to acidic soil (Clark *et al.*, 1995a, 1997a).

Acidic soils amended with FGD+K also benefitted plant growth (M. E. Sumner, University of Georgia, Athens, unpublished data). High Ca (or high soil pH) may reduce P availability (He *et al.*, 1996a,b; Stout *et al.*, 1998, 2000). If sufficient Ca is added to form precipitates or if pH is sufficiently high to inactivate P, deficiencies of P in plants may occur. This disorder occurred consistently when corn was grown in acidic soil amended with many FGDs (R. B. Clark, USDA-ARS, Beaver, WV, personal observations). Risks of imbalanced ratios of the essential nutrients Mg and K occur mainly when plants are grown in acidic soils, while P deficiency may occur in both low and high pH soils.

5.4. Boron toxicity

Boron is a soluble mineral nutrient contained in many materials added to FGD for stabilization, and B toxicity can be a problem for plants grown in soils amended with FGDs containing high B. FAs used to stabilize FGDs often are rich in B (Table 4). Although B is essential to plants, differences between sufficiency and toxicity are narrow (Marschner, 1995). Boron is also water soluble and leaches readily. After FGD-stabilized materials have been leached, B toxicity is commonly alleviated. Boron toxicity in field-grown plants may occur soon after FGDs have been applied to soil, but the toxicity was alleviated once rains leached B from soil (Clark *et al.*, 1999a; Zaifnejad *et al.*, 1998). Plants also vary in susceptibility to B toxicity. Only low levels of stabilized FGD should be applied to sensitive crops such as cherry, peach, and kidney beans (USEPA, 2008c). Alfalfa specifically needs relatively high levels of B for optimum growth, as do apple and pear trees, while corn, cereal crops, and some trees are relatively susceptible to B toxicity (Marschner, 1995). When FGDs containing FA are added to soil, the application rate needs to be matched to the B requirement/sensitivity of the crop.

5.5. Excessive accumulation of nutrients in plants

Control of FGD application rates is required to prevent plant accumulation of excess concentrations of mineral elements. Since FGDs contain high Ca and S levels (Table 2), both elements could accumulate in plants at excessive concentrations. Calcium can interact with several mineral nutrients to induce mineral disorders/deficiencies or accumulate in excess. Young corn plants grown in acidic soil with nonstabilized and stabilized FGDs did not have excessive leaf Ca concentrations (>10–15 g kg^{-1}) even though these FGDs contained high levels of Ca (Clark *et al.*, 1999b). However, leaf S concentrations were near excess (>5.0 g kg^{-1}) when plants were grown with comparable levels of several FGDs (Clark *et al.*, 1999b). Higher plant S was reported for alfalfa and bermudagrass grown in field soils amended with FGDs than in unamended plots, but S concentrations did not reach values considered hazardous to animal intake (4.0–4.5 g kg^{-1}) (Dorsett *et al.*, 1995; Stout and Priddy, 1996).

5.6. Induced Al toxicity

Ca^{2+} readily exchanges with Al^{3+} and other positively charged Al species on soil particle exchange sites (Foy, 1992). Because Al becomes more available and potentially more toxic to root growth at low soil pH (Kinraide, 1991), Ca^{2+} from FGDs exchange with and increase soil solution Al^{3+} to induce Al toxicity in soil if pH has not risen sufficiently to inactivate

Al-ions. Toxic forms of Al also are inactivated by high Ca and S levels (Foy, 1992). Aluminum toxicity induced by Ca on corn occurred when $< 5\%$ by weight of highly pure (chemical grade) $CaSO_4$ was added to acidic soil, but once $CaSO_4$ was added at $> 5\%$ by weight, growth inhibition was alleviated even though soil pH remained similar (Clark *et al.*, 1995b). Because agricultural rates of FGD gypsum application will be far below these levels, the likelihood of induced Al toxicity is minimal.

5.7. Sulfite toxicity

Scrubber sludge FGD may contain high levels of sulfite (Table 2). Even low levels of sulfite are toxic to plants (Bertelsen and Gissel-Nielsen, 1987; Clark *et al.*, 1995b), so use of high sulfite FGDs may be detrimental to plants unless sulfite is eliminated or reduced. Oxidizing sulfite to sulfate is an effective means of eliminating sulfite. Sulfite is converted to sulfate in soil relatively rapidly (within days or weeks) when exposed to oxygen from air (Bertelsen and Gissel-Nielsen, 1988; Ritchey *et al.*, 1995b). Under normal conditions, sulfite from FGDs spread on land during the off-season or sufficiently early before planting would be oxidized by time of planting. In soils with low pH, sulfite may also form SO_2 which is highly toxic to plants/insects (Ritchey *et al.*, 1995b). When considering use of high $CaSO_3$ FGDs that have been oxidized to sulfate, which makes them gypsum products, information about gypsum use on land would be applicable (Alcordo and Rechcigl, 1993, 1995; Korcak, 1998b; Miller, 1995; Ritchey and de Sousa, 1997; Ritchey *et al.*, 1995a, 1997, 2000; Shainberg *et al.*, 1989).

5.8. Trace element toxicity

Probably the major concern for use of FGDs and other CCPs on agricultural land is the potential hazards associated with trace element (As, B, Cd, Cr, Cu, Hg, Mo, Ni, Pb, Se, Zn) buildup in soil, water (surface and ground), plants, animals and humans, and organisms, particularly those entering the food/feed chain. Limitations have been established (Table 6) for concentration levels of trace elements in drinking water (USEPA, 2008a), total land loading (CWA-503 limits; USEPA, 1994), and TCLP leachates (USEPA 2008b). Concentrations of trace elements found in soils and plants and their critical total accumulation are listed in Table 7. Concentrations of trace elements added to land, regardless of source, should be monitored and be within established standard limits (Tables 6 and 7). Analysis of trace elements in FGD materials should be made available to agricultural users prior to land application. When FGDs are applied to land at agronomic rates the major trace element concerns are Hg, As, and Se (USEPA, 2008c; Korcak, 1995, 1998a; Wright *et al.*, 1998). Arsenic, in particular, may be elevated in FGDs

Table 7 Normal and critical total concentration ranges of trace elements in soils and plants[a]

Element	Normal in soil (mg kg^{-1})	Critical total in soil (mg kg^{-1})	Normal in foliage (mg kg^{-1})	Critical total in foliage (mg kg^{-1})
Ag	0.01–8	2	0.1–0.8	1–4
As	0.1–40	20–50	0.02–7	1–20
Au	0.001–0.002	–	0.0017	<1
B	2–150	–	10–100	>100
Ba	70–3000	–	–	500
Be	–	–	<1–7	10–50
Cd	0.01–2.0	3–8	0.1–2.4	4–200
Co	0.1–70	25–50	0.02–1	4–50
Cr	1–1500	75–100	0.03–14	2–30
Cu	2–250	60–125	3–20	5–100
F	<10–4000	–	–	50–500
Hg	0.008–1.11	0.3–5	0.005–0.17	1–8
Li	–	–	3	5–50
Mn	7–10,000	1000–3000	20–2000	100–7000
Mo	0.1–40	2–10	0.03–5	10–50
Ni	0.4–1000	100	0.02–5	8–220
Pb	1.5–300	100–400	0.2–20	30–300
Sb	0.2–10	5–10	0.0001–0.2	1–2
Sc	0.8–20	–	–	–
Se	0.1–5	5–10	0.001–2	3–40
Sr	5–1000	–	–	–
Sn	1–200	50	0.2–6.8	60–63
Ti	0.03–24,000	–	–	50–200
Tl	0.1–0.8	1	0.03–3	20
U	0.7–9	–	0.005–0.06	–
V	0.7–500	5–100	0.001–1.5	1–13
W	0.5–83	–	0.005–0.15	–
Zn	1–900	70–400	1–400	100–900
Zr	–	–	–	15

[a] From Alcordo and Rechcigl (1995), Alloway (1990), Bilski *et al.* (1995), Kabata-Pendias and Pendias (1992), and Stout *et al.* (1988). Concentrations at or above the critical level can have a detrimental impact on plant growth.

containing added FA (Miller and Miller, 2000). Arsenic is toxic to plants and is especially toxic to animals. The chemistry of As is similar to that of P.

Mercury loss from coal-fired power plants, as a gas or in CCPs, is a major environmental concern. The amount of Hg in FGD materials is <1.2 mg kg^{-1} with a range of 0.14–1.17 mg kg^{-1} (Schroeder and Kairies, 2005); however, amounts and types of trace metals in FGD gypsum can vary

between power plants and between coal sources. Mercury concentrations in FGD gypsum are lower than Hg levels found in other CCP's such as FA's (Table 4) and in fertilizers, sewage sludge, and manures (Table 5). Mercury concentrations of FGD's are far below the limit set by CWA-503 for trace elements in sewage sludge (Table 6). Pflughoeft-Hassett *et al.* (2006) noted that Hg associated with CCPs is stable and it is highly unlikely that it is released under most management conditions. Concentrations of Hg in leachates from FGD materials using either the TCLP (toxicity characteristic leaching procedure) or SGLP (synthetic groundwater leaching procedure) are low and require specialized instrumentation for their detection (Pflughoeft-Hassett *et al.*, 2006). Even though the amount of Hg introduced to the environment from land application of FGD gypsum appears to be extremely low, there is still a concern that FGD gypsum contains Hg that would otherwise escape the smoke stack as a vapor. Research currently is being conducted to determine if FGD gypsum contains more Hg than mined gypsum and if this additional Hg poses an environmental threat.

5.9. Miscellaneous constraints

To be widely adopted for agricultural uses, FGDs will need to have several characteristics: (1) low concentration and bioavailability of toxic trace elements, (2) physical and chemical properties that will allow them to be readily used to address agricultural problems, (3) be widely available in large quantities, (4) be consistent in composition from one batch to another so performance is predictable, and (5) be cost-effective to the agricultural user. Some of the FGD materials meet most of the conditions. FGD gypsum materials have been used to improve soil conditions and enhance crop growth. FGDs are produced at power plants around the country. In some cases, such as the southeastern United States, power plants are located near agricultural areas where the products are needed. In other situations, transportation costs would be a significant issue. In general, FGD gypsum has a fairly consistent composition. The composition of other FGDs is more likely to change with operating conditions and raw material inputs. FGDs have a lower cost than mined gypsum, lime, and commercial fertilizer sources.

Wide adoption of FGDs for agricultural uses also depends on development of management practices for specific uses of FGDs (Huang and Lu, 2000). Finally, state and federal regulations will determine which of the FGDs can be used in agriculture. Some states allow land application of FGD gypsum. In the past USEPA promoted beneficial agricultural uses of FGD gypsum. However, USEPA regulations currently under consideration may classify some FGDs containing FA as hazardous waste, thereby stopping their land application.

6. FGD Use for Soil and Crop Management

6.1. Soil and crop response to land application of FGD[2]

Field trials involving land application of FGDs have been conducted on multiple soils and crops under different climatic conditions. Information from these investigations and studies that are currently underway will be used to: (1) identify constraints and cautions for FGD product application to soils and (2) develop management practices to correct problems that have been identified. The following paragraphs will illustrate various uses of FGDs including amelioration of soil acidity, nutrient source for crops, improve soil physical properties, and remove contaminants from water.

Rates of FGDs application vary extensively depending on purpose. For example, rates less than 1 ton acre^{-1} have been applied in some cases and over 5 tons acre^{-1} in others when FGDs have been used to supply fertilizer mineral nutrients like Ca, S, and B to plants (Alva *et al.*, 1999c; Dorsett *et al.*, 1995; Fisher *et al.*, 1997; Sloan *et al.*, 1997). Rates of FGD as high as 10–30 tons acre^{-1} have been used as soil liming agents and/or as gypsum amendments (Dick *et al.*, 2000; Stehouwer *et al.*, 1999; Toma *et al.*, 1999). Application rates greater than 200 tons acre^{-1} have been used when FGDs were added to mine reclamation sites to increase pH of coal/refuse materials to fairly high values (Stehouwer *et al.*, 1998).

Since many FGD materials contain considerable amounts of alkalizing agents FGDs have been used to neutralize acidic soils and alleviate acid soil problems. The amount of FGD product to be applied to land for this purpose can be determined by matching soil lime requirement with total neutralizing potential of FGDs to meet target soil pH values. Total neutralizing potential is based on CCE values. These requirements can be readily obtained through soil testing laboratories, and analytical procedures for this trait are available (Thomas, 1996; Sims, 1996). For example, if the desired target soil pH requires 2 tons $CaCO_3$ acre^{-1} and the CCE of the FGD to be applied is 50%, then double the amount or 4 tons FGD acre^{-1} would be needed to fulfill the soil lime requirement. Stabilized FGDs often have CCE values in this range. FGDs with CCE values near or less than 10% generally are not used to fulfill soil lime requirements because too large an application rate would be required.

The following examples represent crop and soil responses to FGD application. Use of FGD gypsum at 0.25 and 0.5 tons acre^{-1} on two fine sandy loam soils (pH 5.0) increased coastal bermudagrass forage yield by an

[2] Conversion of percentage to land area: Amounts of material used or added to soil are often reported differently in the literature. To convert percentage values added to soil to U.S. ton acre^{-1} multiply value by 10 and to metric tons ha^{-1} multiply by 22. A material application rate of 1% = 10 U.S. ton acre^{-1} = 22 metric ton ha^{-1}.

average of 27% and 36% above controls (no added FGD gypsum) over 2 years (Dorsett *et al.*, 1995). Increases in yield were attributed to increased S as soil S was low. In a study with citrus grown on sandy soil with low extractable Ca at two locations, increased fruit yields, fruit soluble solids, and leaf Ca (one location) were obtained when FGD gypsum was added at 1.0 but not at 0.5 tons acre^{-1} (Alva *et al.*, 1999c). Increases in alfalfa yield were noted when plants were grown in unlimed soil receiving added FGD. The FGD gypsum additions had long-term positive effects and improved subsoil properties for growth of both corn and alfalfa (Toma *et al.*, 1999).

A stabilized FGD (FBC product) containing added Mg [as $CaMg(CO_3)_2$] with a CCE value of 60% was applied at varied levels (0, 0.5, 1.0, and 2.0 the soil lime requirement) to soils at three separate locations in Ohio to test responses of alfalfa and corn to the amendment (Dick *et al.*, 2000). The highest level applied (two times soil lime requirement) was 31 ton acre^{-1}. Alfalfa yields increased slightly, but corn did not the first year after application compared to no added amendment. Alfalfa had enhanced B concentrations, but at concentrations well below phytotoxic levels, and tissue concentrations of Al and Mn decreased in both alfalfa and corn. This FGD product was an effective soil liming material when applied at the standard lime requirement test level, and maintained near neutral soil pH. Surface application of this FGD product enhanced downward movement of Mg, Ca and S to subsoil. Effects of surface application of this FGD product on subsoil chemistry depended on subsoil Ca status. That is, subsoil with high Ca status had greater depletion of Ca and increased Al compared to subsoil with lower Ca status. Surface movement of Mg and S decreased subsoil Al and increased subsoil Mg.

An FGD product was tested (Sloan *et al.*, 1999), to determine whether it could serve as a nutrient and lime source for establishment of alfalfa. Alfalfa is grown as a perennial crop for 3–5 years before crop rotation, and soil is adjusted to pH 7 or greater when the crop is established. In addition, both B and S fertilizers are needed for maximum crop production. Application of an FGD product containing 50% limestone equivalent in addition to moderate levels of B and S was appropriate to produce alfalfa when applied at the lime requirement rate. Elements such as B and S, which would be supplied in excess if the FGD were applied at disposal rates, became important fertilizers that improved yield and quality of the alfalfa for feeding livestock (Sloan *et al.*, 1999).

Field trials (Dick, 2008; Larrimore, 2008) were conducted on Coastal Plain soils in the southeastern U.S. to determine whether FGD gypsum would be an effective substitute for mined gypsum in peanut production systems. Gypsum must be applied to the coarse textured soils of the Coastal Plain region to support grain filling of peanuts. Application of FGD gypsum at a few tons acre^{-1} fully satisfies the Ca requirement of peanuts at all of the

production sites. FGD gypsum also improves crop production and crop quality for a number of other crops including tomatoes and corn.

Field trials were conducted in Mississippi (Rhoton *et al.*, 2010) to determine the effects of FGD gypsum on soil properties and erosion in the southeastern United States. Soils in this part of the country are of poor quality and adjacent water bodies are impaired by sediment and nutrients. Approximately 11 million acres of agricultural land in the southeastern United States are already eroded or are highly susceptible to erosion. FGD gypsum applied at the rate of 3 tons acre^{-1} to a no-till cotton production site significantly improves soil aggregation and limited erosion. FGD gypsum application is compatible with widely used reduced tillage practices because the high solubility of FGD gypsum allows it to be effective even if it is applied only to the soil surface without disturbance. Results show that FGD gypsum improves rainfall infiltration, decreases runoff, and reduces erosion on a degraded soil with high sodium content. Improved water infiltration and storage would be a major benefit to crop production, especially in an area like the southeastern United States which is subject to sever droughts in some years. In many cases, a 15% improvement in rainwater infiltration following FGD gypsum application could help overcome the crop water deficit caused by drought.

Many agricultural areas in the United States require surface (ditches) or subsurface (tile drains) artificial drainage systems to control the height of the water table so agricultural production can occur. However, these drainage systems can serve as conduits for movement of contaminants from agricultural fields to sensitive bodies of water (e.g., Chesapeake Bay, Gulf of Mexico). Technologies are being developed using FGDs and other by-products to remove nutrients, trace elements, pesticides, and hormones from surface and subsurface drainage systems. Two experiments were conducted on the Delmarva Peninsula (Bryant *et al.*, 2010) to remove soluble P from water in or moving toward surface drainage ditches that flow into the Chesapeake Bay. In the first experiment, a ditch filter containing FGD gypsum precipitated from 35% to 90% of the soluble P from ditch flow that passed through the filter. In the second experiment, FGD gypsum-filled trenches installed parallel to drainage ditches removed 50–95% of the soluble P in lateral groundwater flow moving through the trench. These techniques can substantially reduce the movement of P or other pollutants to sensitive bodies of water such as the Chesapeake Bay.

6.2. Safe and effective use of FGDs

Only those FGDs that do not pose unacceptable risks to public health or the environment can be considered for agricultural uses. To achieve this goal benefits and risks associated with agricultural use of individual FGDs need to be determined. Guidelines for specific agricultural uses of FGDs need to be

developed and tested, and the recommendations need to be transferred to famers. In addition, decision tools are needed to support management, policy and regulatory decisions relative to agricultural uses of FGDs.

Considerable research information is available about FA and FBC use on agricultural land (Bilski *et al.*, 1995; Korcak, 1998a; Stout *et al.*, 1988), but information on FGD materials is more limited. Guidelines for use of FBCs on agricultural land were released in 1988 (Stout *et al.*, 1988), and these guidelines have been followed extensively for use of other CCPs, including FGDs. It is important that information about FGDs be brought together so guidelines for their use can be developed. One major gap is information about long-term effects of land application of FGDs.

Land application rates are a major factor in determining environmental risks associated with agricultural uses of FGDs. In general, application rates of FGDs have been in the range of a few tons $acre^{-1}$ or less and have been based on agronomic needs (e.g., nutrient supply, lime requirement). Extremely high rates of FGDs (100 or more tons $acre^{-1}$) generally have been associated with reclamation of land disturbed by mining or other industrial activities. Disposal of FGDs on agricultural land at high application rates is not an acceptable practice.

In the past, when concentrations of trace elements were reported in soils or plants grown in soil amended with FGDs, they usually were at or below established standards and often were below detection limits (Alva *et al.*, 1999a,b; Clark *et al.*, 1999c; Punshon *et al.*, 1999; Stehouwer *et al.*, 1999).

Accumulation of trace elements over time might be of concern if amendments are added frequently or at sufficiently high levels over a considerable length of time. Table 8 provides an example of how much As would be added to land if amendments were applied at different rates over 50 years. The rates of FGD added to soil in this example were based on

Table 8 Assumed cumulative loading of As that could result from FGD gypsum application to soil over 50 years if added annually (hypothetical assumption)[a]

	Annual rate of FGD application (metric tons ha^{-1})				
Amount of As in FGD (mg kg^{-1})	0.55	1.1	1.6	2.2	4.4
	(kg ha^{-1})				
25	0.7	1.4	2.1	2.8	5.6
50	1.4	2.8	4.2	5.6	11.2
75	2.1	4.2	6.3	8.4	16.8
100	2.8	5.6	8.2	11.2	22.4

[a] Rate of annual FGD application is based on Ca requirement and to some extent on soil lime requirement for peanut.

what might be used for Ca requirements and soil lime requirement for production of peanut (a plant that requires high soil Ca to form pegs of high quality). Assuming that 2.2 tons ha^{-1} (1.0 U.S. tons $acre^{-1}$) of FGD containing high As (100 mg As kg^{-1} product) were applied annually over 50 years, the soil would receive a total of 11.2 kg As ha^{-1}. Since 1.0 ha of land is equivalent to 2.2 million kg soil (top 15 cm or 6 in.), this would amount to an accumulation of 0.45 mg As kg^{-1} soil during the 50 years of FGD application. Similar calculations could be made for other trace elements to determine amounts of trace element that would be applied over given length of time.

Additional research is needed to assess the long-term effects of FGD use in agriculture. For example, new technology to reduce greenhouse gases and remove sulfur dioxide, Hg, and particulates from power plant emissions may increase trace elements such as Hg and As in FGDs. The use of these FGDs may create risks to the environment depending on coal source, technology used, and land application rate.

7. Conclusions

Annual production of FGDs will increase considerably as more coal-fired power plants come on-line and as SO_2 scrubbers are added to existing power plants to comply with EPA Clean Air Interstate Rules. FGDs could have a number of beneficial uses in agriculture: they serve as a nutrient source for crops; remediate sodic and acidic soils; improve soil chemical and physical properties to increase water infiltration and storage in soils and reduce runoff and soil erosion; and reduce movement of sediment, nutrients, and agricultural chemicals to surface water. FGD gypsum seems to be the FGD with the greatest promise of providing agricultural benefits with minimal negative impacts on the environment. There are significant areas of degraded soils in the United States that could benefit from application of FGD gypsum.

Benefits and risks for specific agricultural uses of FGD gypsum will need to be determined and guidelines for these specific uses developed. Questions about the risks associated with Hg and As levels in FGD gypsum will need to be resolved. The results of research to quantify benefits and risks for specific uses of FGD materials should be the basis for acceptance or rejection of future agricultural uses of these materials. Development of new uses for FGDs in agriculture will reduce the amount of FGDs that need to be disposed of or sequestered long-term in landfills or impoundment ponds.

ACKNOWLEDGMENTS

We thank Drs W. Dick, C. D. Foy, K. D. Ritchey, and M, Elrashidi for their excellent review of the manuscript and valuable suggestions.

APPENDIX

Scientific names of plant species mentioned in text

Common name	Scientific name
Alfalfa	*Medicago sativa* L.
Apple	*Malus* spp.
Barley	*Hordeum vulgare* L.
Bermudagrass	*Cynodon dactylon* (L.) Pers.
Citrus	*Citrus* spp.
Clover, white	*Trifolium repens* L.
Corn (maize)	*Zea mays* L.
Cotton	*Gossypium hirsutum* L.
Fescue, tall	*Festuca arundinacea* Schreb.
Gamagrass, eastern	*Tripsacum dactyloides* (L.) L.
Orchardgrass	*Dactylis glomerata* L.
Peanut	*Arachis hypogaea* L.
Pear	*Pyrus* spp.
Radish	*Raphanus sativus* L.
Sorghum	*Sorghum bicolor* (L.) Moench
Soybean	*Glycine max* (L.) Merr.
Switchgrass	*Panicum virgatum* L.
Sycamore	*Platanus occidentalis* L.
Wheat	*Triticum aestivum* L.

REFERENCES

ACAA (American Coal Ash Association). (1990–2009). Coal Combustion Product (CCP) Production and Use Surveys. ACAA, Alexandria, VA, Now at Aurora, CO. http://www.acss-usa.org/CCPSurveyShort.htm. Oct. 5, 2009.

Alcordo, I. S., and Rechcigl, J. E. (1993). Phosphogypsum in agriculture: A review. *Adv. Agron.* **49,** 55–118.

Alcordo, I. S., and Rechcigl, J. E. (1995). Phosphogypsum and other by-product gypsums. *In* "Soil Amendments and Environmental Quality" (J. E. Rechcigl, Ed.), pp. 365–425. Lewis Publishers, Boca Raton, FL.

Alloway, B. J. (1990). Heavy Metals in Soils. John Wiley, New York.

Alva, A. K., Bilski, J. J., Sajwan, K. S., and van Clief, D. (1999a). Leaching of metals from soils amended with fly ash and organic byproducts. *In* "Biogeochemistry of Trace Elements in Coal and Coal Combustion Byproducts" (K. S. Sajwan, A. K. Alva, and R. F. Keefer, Eds.), pp. 193–222. Kluwer Academic/Plenum Publishers, New York.

Alva, A. K., Paramsivam, S., Prakash, O., Sajwan, K. S., Ornes, W. H., and van Clief, D. (1999b). Effects of flyash and sewage sludge amendments on transport of metals in different soils. *In* "Biogeochemistry of Trace Elements in Coal and Coal Combustion Byproducts" (K. S. Sajwan, A. K. Alva, and R. F. Keefer, Eds.), pp. 207–222. Kluwer Academic/Plenum Publishers, New York.

Alva, A. K., Zhu, B., Hostler, H. K., and Obreza, T. A. (1999c). Citrus tree growth and fruit production response to flue-gas desulfurization gypsum amendment in sandy soils. *In* "Biogeochemistry of Trace Elements in Coal and Coal Combustion Byproducts" (K. S. Sajwan, A. K. Alva, and R. F. Keefer, Eds.), pp. 293–307. Kluwer Academic/ Plenum Publishers, New York.

Bertelsen, F., and Gissel-Nielsen, G. (1987). Toxicity of root-applied sulphite in Zea mays. *Environ. Geochem. Health* **9,** 12–16.

Bertelsen, F., and Gissel-Nielsen, G. (1988). Oxidation of sulphite originating from flue gas desulfurization waste in soil. *Environ. Geochem. Health* **10,** 26–30.

Bilski, J. J., Alva, A. K., and Sajwan, K. S. (1995). Fly ash. *In* "Soil Amendments and Environmental Quality" (J. E. Rechcigl, Ed.), pp. 327–363. Lewis Publishing, Boca Raton, FL.

Brown, S., Angle, J. S., and Jacobs, L. (1998). Beneficial Co-utilization of Agricultural, Municipal and Industrial By-products. Kluwer Academic Publishers, Boston, MA.

Bryant, R., Buda, A., Kleinman, PP, Church, C., Mcgrath, J., Grubb, K., and Bose, S. (2010). Using FGD gypsum to remove soluble phosphorus from agricultural drainage waters. "Agronomy Abstracts", American Society of Agronomy, Madison, WI, pp. 270.

Butalia, T. S., Wolfe, W. E., and Dick, W. A. (1999). Developments in utilization of CCPs in Ohio. "Proceedings of 13th International Symposium on Use and Management of Coal Combustion Products (CCPs)", pp. 80-1–80-13. Electric Power Research Institute, Palo Alto, CA.

Chen, Y., and Barak, P. (1982). Iron nutrition of plants in calcareous soils. *Adv. Agron.* **35,** 217–240.

Chen, L., Dick, W. A., and Nelson, S. Jr. (2005). Flue gas desulfurization products as sulfur sources for alfalfa and soybean. *Agron. J.* **97,** 265–271.

Chen, L., Kost, D., and Dick, W. A. (2008). Flue gas desulfurization products as sulfur sources for corn. *Soil Sci. Soc. Am. J.* **77,** 1464–1470.

Chichilo, P., and Whittaker, C. W. (1961). Trace elements in agricultural limestone of the United States. *Agron. J.* **53,** 139–144.

Chun, S., Nishiyama, M., and Matsomto, S. (2007). Response of corn growth in salt-affected soils of northeast China to flue-gas desulfurization by-product. *Commun. Soil Sci. Plant Anal.* **38,** 813–825.

Clark, R. B., Zeto, S. K., Ritchey, K. D., Wendell, R. R., and Baligar, V. C. (1995a). Coal combustion by-product use on acid soil: Effects on maize growth and soil pH and electrical conductivity. *In* "Agricultural Utilization of Urban and Industrial By-Products" (D. L. Karlen, R. J. Wright, and W. D. Kemper, Eds.), pp. 131–155. American Society of Agronomy, Madison, WI, Special Publication No. 58.

Clark, R. B., Zeto, S. K., Ritchey, K. D., Wendell, R. R., and Baligar, V. C. (1995b). Effects of coal flue gas desulfurization combustion by-products and calcium sulfite, sulfate, and carbonate on maize grown in acid soil. *In* "Plant-Soil Interactions at Low pH: Principles and Management" (R. A. Date, N. J. Grundon, G. E. Rayment, and M. E. Probert, Eds.), pp. 519–525. Kluwer Academic Publishers, New York.

Clark, R. B., Zeto, S. K., Ritchey, K. D., and Baligar, V. C. (1997a). Growth of forages on acid soil amended with flue gas desulfurization by-products. *Fuel* **74,** 771–775.

Clark, R. B., Zeto, S. K., Ritchey, K. D., and Baligar, V. C. (1997b). Maize growth and mineral acquisition on acid soil amended with flue gas desulfurization by-products and magnesium. *Commun. Soil Sci. Plant Anal.* **28,** 1441–1459.

Clark, R. B., Zeto, S. K., Ritchey, K. D., and Baligar, V. C. (1999a). Boron accumulation by maize grown in acidic soil amended with coal combustion products. *Fuel* **78,** 179–185.

Clark, R. B., Zeto, S. K., Ritchey, K. D., and Baligar, V. C. (1999b). Calcium and sulfur concentrations in leaves of maize grown in acidic soil amended with coal combustion products. "Proceedings of the 13th International Symposium on Use and Management of Coal Combustion Products (CCPs)", Vol. 1, pp. 15-1–15-11. Electric Power Research Institute, Palo Alto, CA.

Clark, R. B., Zeto, S. K., Ritchey, K. D., and Baligar, V. C. (1999c). Nickel, lead, cadmium, and chromium concentrations in shoots of maize grown on acid soil amended with coal combustion by-products. *In* "Biogeochemistry of Trace Elements in Coal and Coal Combustion Byproducts" (K. S. Sajwan, A. K. Alva, and R. F. Keefer, Eds.), pp. 259–268. Kluwer Academic/Plenum Publishers, New York.

Clark, R. B., Ritchey, K. D., and Baligar, V. C. (2001). Benefits and constraints for use of FGD products on agricultural land. *Fuel* **80,** 821–828.

Coal Combustion By-product Information Network Website. (1999). http://www.osmre.gov (technology and development, CCB information).

College, J. W., Beeghly, J. H., Ritchey, K. D., Baligar, V. C., and Clark, R. B.(Inventors). (1997). Method of ameliorating acid soil to enhance plant growth.Patent No. 5, 628, 811 (May 13, 1997). Dravo Lime Co., Pittsburgh, PA and U.S. Department of Agriculture, Beltsville, MD (assignees).

DeSutter, T. M., and Cihacek, L. J. (2009). Potential agricultural uses of flue gas desulfurization gypsum in the northern Great Plains. *Agron. J.* **101,** 817–825.

Dick, W. A. (2008). Land application of FGD gypsum for agricultural use: What do we know!. Beneficial Use of Industrial Material Summit, Denver, CO, http://www.beneficiausesummit.com/2008/presentations/WarrenDick.pdf.

Dick, W. A., Stehouwer, R., Bigham, J. M., Wolfe, W. E., Hao, Y., Adriano, D. C., Beeghly, J. H., and Haefner, R. J. (2000). Beneficial uses of flue gas desulfurization by-products: Examples and case studies of land application. *In* "Land Application of Agricultural, Industrial, and Municipal By-Products" (J. F. Power and W. A. Dick, Eds.), pp. 505–536. Soil Science Society of America, Madison, WI, Book Series No. 6.

Dick, W. A., Kost, D., and Nakano, N. (2006). A Review of Agricultural and Other Land Application Uses of Flue Gas Desulfurization Products. Electric Power Research Institute, Palo Alto, CA Report 101385.

Dorsett, D. J., Nickel, L., Pennington, H. D., and Calloway, D. (1995). Preliminary evaluation of coal generated by-product gypsum as a soil amendment on improved hybrid bermudagrass. "Proceedings of the 11th International Symposium on Use and Management of Coal Combustion Products (CCPs)", Vol. 1, pp. 10-1–10-17. Electric Power Research Institute, Palo Alto, CA.

Farina, M. P. W., Channon, P., and Thibaud, G. R. (2000a). A comparison of strategies for ameliorating subsoil acidity. I. Long-term growth effects. *Soil Sci. Society Am. J.* **64,** 646–651.

Farina, M. P. W., Channon, P., and Thibaud, G. R. (2000b). A comparison of strategies for ameliorating subsoil acidity. II. Long-term soil effects. *Soil Sci. Soc. Am. J.* **64,** 652–658.

Fisher, B., Blackstock, T., and Franciosi, F. (1997). Production and marketing of agricultural products from FGD residues. "Proceedings of the 1997 International Ash Utilization Symposium", pp. 78–84. University of Kentucky, Lexington.

Foy, C. D. (1992). Soil chemical factors limiting plant root growth. *Adv. Soil Sci.* **19,** 87–149.

Graham, R. D., Hannam, R. J., and Uren, N. C. (1988). Manganese in Soils and Plants. Kluwer Academic Publishers, New York.

He, Z. L., Baligar, V. C., Martens, D. C., Ritchey, K. D., and Kemper, W. D. (1996a). Kinetics of phosphate rock dissolution in acidic soil amended with liming materials and cellulose. *Soil Sci. Soc. Am. J.* **60,** 1587–1595.

He, Z. L., Baligar, V. C., Martens, D. C., Ritchey, K. D., and Kemper, W. D. (1996b). Factors affecting phosphate rock dissolution in acid soil amended with liming materials and cellulose. *Soil Sci. Soc. Am. J.* **60,** 1596–1601.

Huang, W. Y., and Lu, Y. C. (2000). Estimating the benefits of agricultural use of municipal, animal, and industrial by-products. *In* "Land Application of Agricultural, Industrial, and Municipal By-Products" (J. F. Power and W. A. Dick, Eds.), pp. 361–386. Soil Science Society of America, Madison, WI, Book Series No. 6.

Kabata-Pendias, A., and Adriano, D. C. (1995). Trace metals. *In* "Soil Amendments and Environmental Quality" (J. E. Rechcigl, Ed.), pp. 139–167. Lewis Publishing, Boca Raton, FL.

Kabata-Pendias, A., and Pendias, H. (1992). Trace Elements in Soils and Plants. CRC Press, Boca Raton, FL.

Kinraide, T. B. (1991). Identity of the rhizotoxic aluminium species. *Plant Soil* **134,** 167–178.

Korcak, R. F. (1995). Utilization of coal combustion by-products in agriculture and horticulture. *In* "Agricultural Utilization of Urban and Industrial By-Products" (D. L. Karlen, R. J. Wright, and W. D. Kemper, Eds.), pp. 107–130. American Society of Agronomy, Madison, WI, Special Publication No. 58.

Korcak, R. F. (1998a). Agricultural uses of coal combustion byproducts. *In* "Agricultural Uses of Municipal, Animal, and Industrial Byproducts" (R. J. Wright, W. D. Kemper, P. D. Millner, J. F. Power, and R. F. Korcak, Eds.), pp. 103–119. U.S. Department of Agriculture, Agricultural Research Service, Beltsville, MD, Conservation Research Report No. 44.

Korcak, R. F. (1998b). Agricultural uses of phosphogypsum, gypsum, and other industrial byproducts. *In* "Agricultural Uses of Municipal, Animal, and Industrial Byproducts" (R. J. Wright, W. D. Kemper, P. D. Millner, J. F. Power, and R. F. Korcak, Eds.), pp. 120–126. U.S. Department of Agriculture, Agricultural Research Service, Beltsville, MD, Conservation Research Report No. 44.

Kosta, D. A., Bingham, J. M., Stehouwer, R. C., Beeghly, J. H., Fowler, R., Traina, S. J., Wolfe, W. E., and Dick, W. A. (2005). Chemical and physical properties of dry flue gas desulfurization products. *J. Environ. Qual.* **34,** 676–686.

Larrimore, L. (2008). Incentives for Use of FGD Gypsum in Agriculture. Beneficial Use of Industrial Material Summit, Denver, CO, http://www.beneficiausesummit.com/2008/presentations/LamarLarrimore.pdf.

Levy, G. J. (2000). Sodicity. *In* "Handbook of Soil Science" (M. E. Sumner, Ed.), pp. G-27–G-63. CRC Press, Boca Raton, FL.

Logan, T. J., and Burnham, J. C. (1995). The alkaline stabilization with accelerated drying process (N-Viro): An advanced technology to convert sewage sludge into a soil product. *In* "Agricultural Utilization of Urban and Industrial By-products" (D. L. Karlen, R. J. Wright, and W. D. Kemper, Eds.), pp. 209–223. American Society of Agronomy, Madison, WI, Special Publication No. 58.

Maas, E. V. (1990). Crop salt tolerance. *In* "Agricultural Salinity Assessment and Management" (K. K. Tanji, Ed.), Manuals and Reports on Engineering Practice No. 71, pp. 262–304. American Society of Civil Engineering, New York.

Marschner, H. (1995). Mineral Nutrition of Higher Plants. Academic Press, San Diego, CA.

McDowell, L. R. (1992). Minerals in Animals and Human Nutrition. Academic Press, San Diego, CA.

Mengel, K., and Kirkby, E. A. (1982). Principles of Plant Nutrition. International Potash Institute, Bern, Switzerland.

Miller, W. P. (1995). Environmental considerations in land application of by-product gypsum. *In* "Agricultural Utilization of Urban and Industrial By-Products" (D. L. Karlen, R. J. Wright, and W. D. Kemper, Eds.), pp. 183–208. American Society of Agronomy, Madison, WI, Special Publication No. 58.

Miller, D. M., and Miller, W. P. (2000). Land application of wastes. *In* "Handbook of Soil Science" (M. E. Sumner, Ed.), pp. G-217–G-245. CRC Press, Boca Raton, FL.

Miller, E. R., Lei, X., and Ullrey, D. E. (1991). Trace elements in animal nutrition. *In* "Micronutrients in Agriculture" (J. J. Mortvedt, R. F. Cox, L. M. Shuman, and R. M. Welch, Eds.), 2nd edn. pp. 593–662. Soil Science Society of America, Madison, WI, Book Series No. 4.

Norton, L. D. (1995). Mineralogy of high calcium/sulfur-containing coal combustion by-products and their effect on soil surface sealing. *In* "Agricultural Utilization of Urban and Industrial By-Products" (D. L. Karlen, R. J. Wright, and W. D. Kemper, Eds.), pp. 87–106. American Society of Agronomy, Madison, WI, Special Publication No. 58.

Norton, L. D., and Zhang, X. C. (1998). Liming to improve chemical and physical properties of soil. *In* "Handbook of Soil Conditioners: Substances that Enhance the Physical Properties of Soil" (A. Wallace and R. E. Terry, Eds.), pp. 309–331. Marcel Dekker, New York.

Norton, L. D., Shainberg, I., Cihacek, L., and Edwards, J. H. (1998). Erosion and soil chemical properties. *In* "Soil Quality and Soil Erosion" (R. Lal, Ed.), pp. 39–56. CRC Press, Boca Raton, FL.

Page, A. L., Elseewi, A. A., and Straughan, I. R. (1979). Physical and chemical properties of fly ash from coal-fired power plants with reference to environmental impacts. *Residue Rev.* **71,** 83–120.

Pflughoeft-Hassett, D. F., and Renninge, S. (1999). Review of barriers to the increased utilization of CCBs by government and commercial sectors. "Proceedings of the 13th International Symposium on the Use and Management of Coal Combustion Products (CCPs)", pp. 79-1–79-13. Electric Power Research Institute, Palo Alto, CA.

Pflughoeft-Hassett, D. F., Sondreal, E. A., Steadman, E. N., Eylands, K. E., and Dockter, B. A. (1999). Barriers to the increased utilization of coal combustion/desulfurization byproducts by government and commercial sectors update 1998.Report to U.S. Department of Energy, Federal Energy Technical Center, Morgantown, WV.

Pflughoeft-Hassett, D. F., Hassett, D. J., Heebink, L. V., and Buckley, T. D. (2006). The Current State of the Science Related to the Re-Release of Mercury from Coal Combustion Products. American Coal Ash Association, Aurora, CO, Ash at Work, Issue 1, pp. 26–27.

Power, J. F., and Dick, W. A. (2000). Land Application of Agricultural, Industrial, and Municipal By-Products. Soil Science Society of America, Madison, WI, Book Series No. 6.

Punshon, T., Knox, A. S., Adriano, D. C., Seaman, J. C., and Weber, J. T. (1999). Flue gas desulfurization (FGD) residue: Potential applications and environmental issues. *In* "Biogeochemistry of Trace Elements in Coal and Coal Combustion Byproducts" (K. S. Sajwan, A. K. Alva, and R. F. Keefer, Eds.), pp. 7–28. Kluwer Academic/Plenum Publishers, New York.

Ransome, L. S., and Dowdy, R. H. (1987). Soybean growth and boron distribution in a sandy soil amended with scrubber sludge. *J. Environ. Qual.* **16,** 171–175.

Rhoton, F., Buehring, N., Essing, M. E., McChesney, D. S., and Tyler, D. D. (2010). Potential use of FGD gypsum in agriculture. "Proceedings of the 32nd Southern

Conservation Agricultural Systems Conference", p. 36, University of Tennessee, Knoxville, TN.

Ritchey, K. D., and de Sousa, D. M. G. (1997). Use of gypsum in management of subsoil acidity in Oxisols. *In* "Plant-Soil Interactions at Low pH: Sustainable Agriculture and Forestry Production" (A. C. Moniz, A. M. C. Furlani, R. E. Schaffert, N. K. Fageria, C. A. Rosolem, and H. Cantarella, Eds.), pp. 165–178. Brazilian Soil Science Society, Campinas/Viçosa, Brazil.

Ritchey, K. D., Feldhake, C. M., Clark, R. B., and de Sousa, D. M. G. (1995a). Improved water and nutrient uptake from subsurface layers of gypsum-amended soils. *In* "Agricultural Utilization of Urban and Industrial By-Products" (D. L. Karlen, R. J. Wright, and W. D. Kemper, Eds.), pp. 157–181. American Society of Agronomy, Madison, WI, Special Publication No. 58.

Ritchey, K. D., Kinraide, T. B., and Wendell, R. R. (1995b). Interactions of calcium sulfite with soils and plants. *Plant Soil* **173,** 329–335.

Ritchey, K. D., Feldhake, C. M., Clark, R. B., Elrashidi, M. A., Baligar, V. C., Stout, W. L., Sharpley, A. N., Norton, L. D., and Korcak, R. F. (1997). Recent developments in agricultural uses of gypsum. *In* "Proceedings of the 5th International Conference on FGD and Synthetic Gypsum" (L. M. Luckevich, V. R. Trimble, and R. E. Collins, Eds.), pp. 20-1–20-20. ORTECH Corporation, Mississauga, Ontario, Canada.

Ritchey, K. D., Elrashidi, M. A., Clark, R. B., and Baligar, V. C. (1998a). Potential for utilizing coal combustion residues in co-utilization products. *In* "Beneficial Co-utilization of Agricultural, Municipal and Industrial By-products" (S. Brown, J. S. Angle, and L. Jacobs, Eds.), pp. 139–147. Kluwer Academic Publishers, Boston, MA.

Ritchey, K. D., Feldhake, C. M., Belesky, D. P., Clark, R. B., Baligar, V. C., and Elrashidi, M. A. (1998b). Lime, fertilizer, and coal combustion byproduct effects on grass production in an acidic Appalachian pasture. "Agronomy Abstracts", American Society of Agronomy, Madison, WI.

Ritchey, K. D., Clark, R. B., Elrashidi, M. A., and Baligar, V. C. (2000). Properties and examples of beneficial use of gypsumlike by-products. *In* "Land Application of Agricultural, Industrial, and Municipal By-Products" (J. F. Power and W. A. Dick, Eds.), pp. 537–565. Soil Science Society of America, Madison, WI, Book Series No. 6.

Robson, A. D. (1993). Zinc in Soils and Plants. Kluwer Academic Publishers, New York.

Schroeder, K., and Kairies, C. (2005). Distribution of Mercury in FGD by Products University of Kentucky, Lexington, KY, World of Coal Ash, April 11–15, 2005.

Shainberg, I., Sumner, M. E., Miller, W. P., Farina, M. P. W., Pavan, M. A., and Fey, M. V. (1989). Use of gypsum on soils: A review. *Adv. Soil Sci.* **9,** 1–111.

Sharpley, A. N., Chapra, S. C., Wedepohl, R., Sims, J. T., Daniel, T. C., and Reddy, K. R. (1994). Managing agricultural phosphorus for protection of surface waters: Issues and options. *J. Environ. Qual.* **23,** 437–451.

Sharpley, A. N., Daniel, T., Sims, T., Lemunyon, J., Stevens, R., and Parry, R. (1999). Agricultural Phosphorus and Eutrophication. U.S. Department of Agriculture, Agricultural Research Service, Washington, DC, ARS-149.

Sims, J. T. (1996). Lime requirement. *In* "Methods of Soil Analysis, Part 3, Chemical Methods" (D. L. Sparks, A. L. Page, P. A. Helmke, R. H. Loeppert, P. N. Soltanpour, M. A. Tabatabai, C. T. Johnston, and M. E. Sumner, Eds.), pp. 491–515. Soil Science Society of America and American Society of Agronomy, Madison, WI, Book Series No. 5.

Sloan, J. J., Dowdy, R. H., Dolan, M. S., and Rehms, G. W. (1997). Field evaluation of flue gas desulfurization residue as a boron and sulfur source for alfalfa. "Proceedings of the International Ash Utilization Symposium", pp. 122–129. University of Kentucky, Lexington, KY.

Sloan, J. J., Dowdy, R. H., Dolan, M. S., and Rehms, G. W. (1999). Plant and soil responses to field-applied flue gas desulfurization residue. *Fuel* **78,** 169–174.

Stehouwer, R. C., Bigham, J. M., and Dick, W. A. (1998). Co-utilization of flue-gas desulfurization and organic by-products for mine reclamation. *In* "Beneficial Co-utilization of Agricultural, Municipal and Industrial By-products" (S. Brown, J. S. Angle, and L. Jacobs, Eds.), pp. 377–395. Kluwer Academic Publishers, New York.

Stehouwer, R. C., Dick, W. A., and Sutton, P. (1999). Acidic soil amendment with a magnesium-containing fluidized bed combustion by-product. *Agron. J.* **91,** 24–32.

Stout, W. L., and Priddy, W. E. (1996). Use of flue gas desulfurization (FGD) by-product gypsum on alfalfa. *Commun. Soil Sci. Plant Anal.* **27,** 2419–2432.

Stout, W. L., Hearn, J. L., Korcak, R. F., and Carlson, C. W. (1988). Manual for Applying Fluidized Bed Combustion Residue to Agricultural Lands. U.S. Department of Agriculture, Agricultural Research Service. U.S. Government Printing Office, Washington, DC, ARS-74.

Stout, W. L., Sharpley, A. N., and Pionke, H. B. (1998). Reducing soil phosphorus solubility with coal combustion by-products. *J. Environ. Qual.* **27,** 111–118.

Stout, W. L., Sharpley, A. N., and Landa, J. (2000). Effectiveness of coal combustion by-products in controlling phosphorus export from soils. *J. Environ. Qual.* **29,** 1239–1244.

Sumner, M. E., Fey, M. V., and Noble, A. D. (1991). Nutrient status and toxicity problems in acid soils. *In* "Soil Acidity" (B. Ulrich and M. E. Sumner, Eds.), pp. 149–182. Springer-Verlag, New York.

Thomas, B. W. (1996). Soil pH and soil acidity. *In* "Methods of Soil Analysis, Part 3, Chemical Methods" (D. L. Sparks, A. L. Page, P. A. Helmke, R. H. Loeppert, P. N. Soltanpour, M. A. Tabatabai, C. T. Johnston, and M. E. Sumner, Eds.), pp. 475–490. Soil Science Society of America and American Society of Agronomy, Madison, WI, Book Series No. 5.

Toma, M., Sumner, M. E., Weeks, G., and Saigusa, M. (1999). Long-term effects of gypsum on crop yield and subsoil chemical properties. *Soil Sci. Soc. Am. J.* **39,** 891–895.

U.S. Geological Survey. (2007). Mineral Year Book, Metals and Minerals (2005), Vol. I. US. Department of the Interior U.S. Geological Survey, Washington, DC, 20402.

U.S. Geological Survey. (2008). Mineral Commodity summaries 2008 Energy Information Administration Update February 2008. http://www.nma.org/statistica-index.asp Aug 21, 2008.

USEPA (U.S. Environmental Protection Agency). (1994). A Guide for Land Appliers on the Requirements of the Federal Standards for the User Disposal of Sewage Sludge. USEPA, Washington, DC, Federal Standards for the Use and Disposal of Sewage Sludge, 40 CFR Part 503. Report 831-B-93-002b (December 1994).

USEPA (U.S. Environmental Protection Agency). (2008a). National Drinking Water Regulations, Maximum Contaminant Level. USEPA, Washington, DC, USEPA, Office of Water, EPA 822-R-96-001 (June 5, 2008).

USEPA (U.S. Environmental Protection Agency). (2008b). Identification and Listing of Hazardous Waste. USEPA, Washington, DC, 40 CFR Part 261 (10-30-08).

USEPA Agricultural uses for flue gas desulfurization (FGD) gypsum. (2008). http://www.epa.gov/osw, U.S. Environmental Protection Agency. EPA530-F08-009 (March 2008).

Wang, S. J., Chen, C. H., Xu, X. C., and Li, Y. J. (2008). Amelioration of alkali soil using flue gas desulfurization products: Productivity and environmental quality. *Environ. Pollut.* **151,** 200–204.

Wolfe, W. E., Butalia, T. S., and Fortner, C. (1999). Preliminary Performance Assessment of an FGD-lined pond facility. *In* "Proceedings of the 13th International Symposium on Use and Management of Coal Combustion Products (CCPs)," pp. 51–1 to 51–17. Electric Power Research Institute, Palo Alto, CA.

Wright, R. J., Codling, E. E., Stuczynski, T., and Siddaramappa, R. (1998). Influence of soil-applied coal combustion by-products on growth and elemental composition of annual ryegrass. *Environ. Geochem. Health* **20,** 11–18.

Zaifnejad, M., Ritchey, K. D., Clark, R. B., Baligar, V. C., and Martens, D. C. (1998). Fluidized bed combustion by-product treatment and leaching of acid soil on growth and boron acquisition of maize. *Commun. Soil Sci. Plant Anal.* **29,** 255–267.

Zeto, S. K., Clark, R. B., Ritchey, K. D., and Baligar, V. C. (1997). Magnesium source and flue gas desulfurization by-product effects on maize growth in acid soil. "Proceedings of the International Ash Utilization Symposium", pp. 70–77. University of Kentucky, Lexington, KY.

Zhou, X., Ritchey, K. D., Clark, R. B., Persud, N., and Belesky, D. P. (2006). Upper profile changes over time in an Appalachian hayfield soil amended with coal combustion by-products. *Commun. Soil Sci. Plant Anal.* **37,** 1247–1267.

CHAPTER THREE

High-Temperature Effects on Rice Growth, Yield, and Grain Quality

P. Krishnan,[*,†,1] B. Ramakrishnan,[‡,2] K. Raja Reddy,[§] *and* V. R. Reddy[*]

Contents

[*] Crop Systems and Global Change Laboratory, USDA-ARS, BARC West, Beltsville, Maryland, USA
[†] Laboratory of Plant Physiology, Central Rice Research Institute, Cuttack, Orissa, India
[‡] Laboratory of Soil Microbiology, Central Rice Research Institute, Cuttack, Orissa, India
[§] Department of Plant and Soil Sciences, Mississippi State University, Mississippi State, Mississippi, USA
[1] Present address: Division of Agricultural Physics, Indian Agricultural Research Institute, New Delhi, India
[2] Present address: Division of Microbiology, Indian Agricultural Research Institute, New Delhi, India

Advances in Agronomy, Volume 111
ISSN 0065-2113, DOI: 10.1016/B978-0-12-387689-8.00004-7

Abstract

Rice (*Oryza sativa* L.) is a globally important cereal plant, and as a primary source of food it accounts for 35–75% of the calorie intake of more than 3 billion humans. With the likely growth of world's population toward 10 billion by 2050, the demand for rice will grow faster than for other crops. There are already many challenges to achieving higher productivity of rice. In the future, the new challenges will include climate change and its consequences. The expected climate change includes the rise in the global average surface air temperature. At the end of the twenty-first century, the increases in surface air temperature will probably be around 1.4–5.8 °C, relative to the temperatures of 1980–1999, and with an increase in variability around this mean. Most of the rice is currently cultivated in regions where temperatures are above the optimal for growth (28/22 °C). Any further increase in mean temperature or episodes of high temperatures during sensitive stages may reduce rice yields drastically. In tropical environments, high temperature is already one of the major environmental stresses limiting rice productivity, with relatively higher temperatures causing

reductions in grain weight and quality. Developing high-temperature stress-tolerant rice cultivars has become a proposed alternative, but requires a thorough understanding of genetics, biochemical, and physiological processes for identifying and selecting traits, and enhancing tolerance mechanisms in rice cultivars. The effects of high-temperature stress on the continuum of soil–rice plant–atmosphere for different ecologies (with or without submerged conditions) also need detailed investigations. Most agronomic interventions for the management of high-temperature stress aim at early sowing of rice cultivars or selection of early maturing cultivars to avoid high temperatures during grain filling. But these measures may not be adequate as high-temperature stress events are becoming more frequent and severe in the future climate. In this review, the effects of high-temperature stress on rice growth, yield, and quality characters, including various morphological, physiological, and biochemical mechanisms along with the possible use of conventional and molecular breeding methods, and crop growth simulation models and techniques are discussed. The mitigation and adaptation strategies for dealing with high-temperature stress in rice are highlighted. We conclude that there are considerable risks for rice production, stemming from high-temperature stress but benefits from the mitigation or adaptation options through progress in rice research may sustain the production systems of rice in the future warmer world.

1. Climate Change and Rice

Intensive research on climate change in recent times shows that rising temperatures may intensify storms, flooding and other severe weather events worldwide, and eventually affect food production. Climate models project that global surface air temperatures may increase by 4–5.8 °C in the next few decades (IPCC, 2007). Increases in temperature will probably offset the likely benefits of increasing atmospheric concentrations of carbon dioxide (CO_2) on crop plants. Seed-producing plants are much more at risk from rising temperatures than are plants cultivated for vegetative parts such as forage plants. Rice is an important global food crop and provides food security for many countries. In the future climatic conditions, the yields of rice would be reduced depending on the growing-season environmental conditions as present-day high temperatures have been implicated to cause reductions in rice yield in many rice-growing areas (Nagarajan *et al.*, 2010; Wassmann *et al.*, 2009a,b; Welch *et al.*, 2010). According to Matsui *et al.* (2001), rice yields in the existing cropping areas could be completely wiped out if most severe climate predications are correct. In the rice-growing regions including those in tropical and subtropical regions, rice has already been cultivated as a summer crop despite relatively high temperatures that occur during its growth cycle (Sung *et al.*, 2003). In these regions, heat stress is a common constraint during anthesis and grain-filling stages (Kobata and

Uemuki, 2004). Because of this constraint at the present time, more so in the predicted future, the need for new heat-tolerant rice cultivars, either identified from the present germplasm or bred, assumes greater significance.

High temperature is known to disrupt water, ion, and organic solute movement across plant membranes, which interferes with photosynthesis and respiration. When subjected to high temperatures, electrolytic leakage may occur from leaves (Halford, 2009). The thermal stability of cell membrane is considered to be positively associated with yield performance. Temperature is a major factor for photosynthesis. But, excessive temperatures can result in a decline in plant leaf photosynthesis and also a decrease in allocation of dry matter to shoots and roots. The adverse effects of high air temperature are not limited to the aboveground portion of rice. As the temperature of floodwater and soil get altered due to high air temperature, the below-ground portion can be equally, if not more affected. This review highlights the global significance of rice, the effects of high temperature due to climate change on growth and development, and yield components of rice and the need for future research studies.

1.1. Rice—an important cereal plant

The historical importance of rice in Asia is so significant that it supported many civilizations in the river deltas of China, India, and Southeast Asia and has become deeply intertwined with the cultures in these regions. Rice has a very wide range of adaptation, growing 3 m below sea level in Kerala in India and more than 3000 m elevation in Nepal and Bhutan. The geographical distribution of rice-growing areas in the world shows that rice is cultivated from 50° North in Central Czechoslovakia and Manchuria in China, on the equator, to 35° South in Uruguay and New South Wales in Australia (Grist, 1986). It is cultivated in regions with more than 3000 mm rainfall, but also in desert regions with less than 50 mm rainfall during its growing season. In the Punjab and Sindh Provinces of Pakistan, rice is cultivated in areas with mean monthly temperatures of more than 33 °C, while the average season temperature in northern Japan is not much higher than 17 °C. For optimum growth and yield, rice requires (i) evenly distributed water during the growing season but relatively dry during the grain-filling period, (ii) temperatures that are sufficiently high throughout the growing season but with somewhat lower night temperatures during the grain-filling period, and (iii) ample solar radiation throughout the growing season. Based on several criteria such as water regime, drainage, soil, and topography, the rice-growing environments are classified into categories such as irrigated, rain fed, upland, and flood prone.

Globally, the rice area harvested has increased only marginally from 120.1 million ha in 1960 to 155.7 million ha in 2008 (Childs and Baldwin, 2010). But, the average rice yield has doubled from 1.84 to

4.25 Mg ha^{-1} during the same period, largely due to the adoption of "Green Revolution" technologies such as the use of high-yielding rice cultivars and application of chemical fertilizers. While the average rice yield under irrigated conditions, particularly in the regions with ample sunshine, is high, the agronomic yields show a high degree of instability and depend on the vagaries of weather and monsoon.

1.2. Climate change and global warming

A very consistent feature of global climate is changes in temperatures and rainfall that vary from year to year and fluctuate widely over a period of time. The atmospheric CO_2 concentration [CO_2] is expected to rise from 380 μmol mol^{-1} currently to between 485 and 1000 μmol mol^{-1} by 2100 (IPCC, 2007). As the consequence of greenhouse effects of many atmospheric trace gases including [CO_2], the warming of Earth may occur. In the past 150 years, the global average surface air temperature has increased significantly by 0.15 ± 0.05 °C per decade (Jones *et al.*, 1999). Due to the projected increases in the concentrations of all greenhouse gases, the expected rise in the global average surface air temperature at the end of the twenty-first century relative to 1980–1999 projected to be around 1.4–5.8 °C (IPCC, 2007). Jones *et al.* (1999) showed that the global surface air temperature rose by 0.57 °C from 1861 to 1901 and by 0.62 °C from 1901 to 1997. In addition, over the period of 1950–1993, nighttime (minimum) temperatures increased at a rate of 0.18 °C per decade, while daytime (maximum) temperatures increased by 0.08 °C per decade in Libya. According to Stanhill (2001) who used the global surface temperature record of the past 140 years, there existed a long and very irregular but generally cool first period between 1860 and 1910, a very rapid, regular, and prolonged period of global warming between 1910 and 1943, an equally long period of small and irregular cooling from 1943 to 1975, and since then, the current warming period thereafter.

The predicted changes include the increases in the mean surface temperatures of Earth by 1.4–5.8 °C by 2100, the decrease in precipitation in the subtropics, and frequent occurrence of extreme events such as flood and drought (IPCC, 2007). The climatic changes are largely driven by increasing atmospheric concentrations of greenhouse gases, stratospheric ozone depletion, aerosol emissions, and land-use changes (Burroughs, 2003). In the past century, the daily minimum (or nighttime) temperature has increased faster than the daily maximum (daytime) temperature (Easterling *et al.*, 1997; Kukla and Karl, 1993). At the International Rice Research Institute (IRRI), Manila, Philippines, Peng *et al.* (2004) has reported a 1.13 °C increase in nighttime temperature over a period of 25 years (1979–2003).

Most of the rice is currently cultivated in regions where temperatures are already above the optimal for growth (28/22 °C); therefore, any further

increase in mean temperature or episodes of high temperatures during sensitive stages of the crop may adversely affect the growth and yield of rice. According to Baker *et al.* (1992), yield decrease was about 7–8% in rice for each 1 °C increase in daytime maximum/nighttime minimum in temperature from 28/21 to 34/27 °C. Sheehy *et al.* (2006) suggested that the interannual climate variability affects rice production and predicted that increasing interannual climate variability would lead to higher yield losses. With the future climate being expected to be highly variable with frequent episodes of stressful temperatures in terms of more numbers of hot days during the crop-growing season, particularly during the reproductive stages of the crop, will adversely affect the growth and yield of rice. Moreover, the increase in temperature will eliminate the likely benefits of projected rise in atmospheric [CO_2] on rice plants (Krishnan *et al.*, 2007). Because of the predicted climate change, the importance of rice as a global crop will grow further as no other cereal crop has the resilience to grow under a wide range of conditions such as flooded to upland conditions, and below sea level to high altitudes.

1.3. Future population increase and demand for rice

The human population in the Earth grew more than 10-fold from about 600 million people in 1700 to 6.79 billion in 2009 (US Census Bureau, World POPClock Projection, 2009). According to the population projections, the world population will continue to grow until at least 2050 (Battisti and Naylor, 2009). Because births outnumber deaths, the human population is expected to reach 10 billion in 2050. The human population will probably be more slowly growing, declining in the more developed regions, more urban, especially in less developed regions, and older than in the twentieth century (Cohen, 2003), and over half of them will live in Asia. The current annual growth rate of global population is 1.22%, which is six times faster in developing nations than in developed regions.

Rice, cultivated as food for direct consumption more so than any other crop, is the second largest consumed cereal after wheat and provides about 80% of the food calorie requirements of more than half of the world's population (FAO, 2008). As the world's population continues to grow toward 10 billion by 2050, the demand for rice will grow faster than for other crops because population growth is greatest in the rice-consuming and rice-producing regions of Asia, Africa, and the America (Dawe, 2007; Easterling *et al.*, 2007). In addition, the shortage of land and water for rice cultivation (Khush, 2005), accompanied by increases in food demand, has also forced cultivation to extend beyond normal monsoon periods, where temperatures are optimal for growth to warmer summer seasons where high temperature is an important constraint. As a summer crop, rice has already

been cultivated in many regions where relatively high temperatures occur during its growth cycle (Sung *et al.*, 2003).

2. Role of Temperature on Growth and Development of Rice

High-temperature stress in plants is a complex function of intensity (temperature in degrees), duration, and rate of increase in temperature (Wahid *et al.*, 2007). The physiological response of plants to temperature stress can be (i) tolerance which is due to mechanisms that maintain high metabolic activity under mild stress and reduced activity under severe stress and (ii) avoidance which involves a reduction of metabolic activity, resulting in a dormant state upon exposure to extreme stress. The latter is not relevant to rice production.

Rice originated in tropical or subtropical areas and is a low-temperature-sensitive crop; crop growth and development are severely damaged below 15 °C. But, extreme temperatures are also destructive to plant growth. Critical temperatures define the environmental conditions under which the life cycle of a rice plant can be completed. Generally, rice is adversely affected by high temperature in the lower elevations of the tropics and by lower temperature in the temperate regions. At different times during the life cycle, rice plant is differentially sensitive to temperature stress. Hence, the critically low and high temperatures, normally below 20 °C and above 30 °C, vary from one growth stage to another (Fig. 1). Critical temperatures differ according to cultivars, duration of critical temperature, diurnal changes, and physiological status of the plant. In this review, the effects of optimal and supraoptimal temperatures on rice plants are highlighted.

2.1. Germination

Rice plants are most tightly linked to the soil water environment from sowing to the establishment of fully functional photosynthetic and water transport systems. Seed germination affects survival and competitiveness across diverse environments. Temperature has a profound influence on germination. As early as 1933, Livingston and Haasis found out that an incubation of 6 days was required for 90% germination at 25 °C, 2 days at 31–36 °C, and an extended period at 0–5 °C. At low temperatures, germination proceeds very slowly and may take a month or longer. Takahashi (1961) examined the effects of temperature on germination using rice seeds of variety Ou-no 200, on three aspects such as temperature, time, and germination percentage. In 2 days, about 90–97% germination was attained under incubation at 27–37 °C. But, the germination

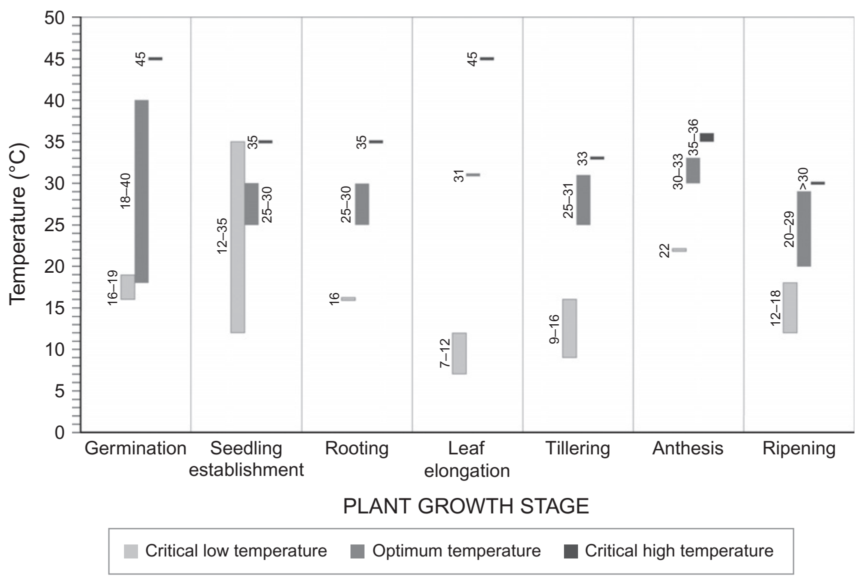


Figure 1 Response of the rice plant to varying temperature at different growth stages (adapted from Yoshida, 1978).

percentage dropped sharply below or above this range. At temperatures between 15 and 37 °C, the incubation time for a germination of 90% or higher was about 6 days. No germination occurred at 8 and 45 °C. The suppression of germination at supraoptimal temperatures is called thermoinhibition. The germinating seeds may experience a 25 °C fluctuation in temperature throughout the course of a day, from a minimum of 22 °C to a maximum of 47 °C over a 12-h period, under upland (aerobic) conditions. Under irrigated conditions, this fluctuation in temperature in a day will be less. If seeds germinate erratically over a long time, seedling growth will not be uniform and plants will mature over a wider period. The freshly harvested seed of rice can have low germination caused by postharvest dormancy, which is referred to as exhibiting "nondeep physiological dormancy" (Hartmann *et al.*, 1997). The postharvest dormancy of rice can be reduced by exposing seed to 3 days of dry heat (50–55 °C) (Roberts, 1965). The IRRI heats all *japonica* and *indica* cultivars to 50 °C for 3 days regardless of their dormancy tendencies.

2.2. Seedling growth

The seedling growth is very sensitive to temperature in the first week of postgermination. The growth rate increases linearly between 22 and 31 °C, suggesting that chemical reactions dominate growth. The enzymatic

breakdown of the seed reserves support more than 70% of growth during this week. After the first week, temperature influences growth less and the relative growth rates are about the same at 25, 28, and 31 °C (Yoshida, 1973). A temperature of 22 °C or below is considered subnormal for seedling growth. The seedling growth may be reasonably good up to 35 °C, above which it declines sharply. The seedlings will die above 40 °C. Nishiyama (1977) reported that the critical minimum temperature for shoot elongation ranged from 7 to 16 °C and that for root elongation from 12 to 16 °C. The critical minimum for elongation of both shoot and root is, hence, about 10 °C. Depending on the cultivars, seed history, and cultural management practices, these critical temperatures may vary.

The elongation of tissue results from two components of cell growth: cell division and cell enlargement. The optimum temperature for cell division of the radicle tip is 25 °C, and that for cell enlargement is 30 °C. The elongation of radicle as a whole, however, is optimum at 30 °C, indicating that cell enlargement dominates division. The elongation of radicle stops below 15 °C and above 40 °C. The temperature quotient (Q_{10}) is used to assess temperature effects on rates of growth and differentiation, which is defined as:

$$Q_{10} = \frac{\text{Rate at}(t+10)^{\circ}\text{C}}{\text{Rate at}\, t^{\circ}\text{C}(2.5)}$$

The use of Q_{10} assumes that rates of differentiation and growth are expected to obey the Arrhenius relation, that is, to increase logarithmically with temperature. For many plant processes, Q_{10} is between 2 and 3 within a moderate temperature range. For the postgermination growth of rice, Q_{10} is about 2, but the relation between growth rate and temperature is linear, not logarithmic. However, the values of Q_{10} normally decrease with increasing temperature. For example, the plant processes such as respiration of rice increase with increasing temperature up to 32 °C, above which it declines. Between 19 and 25 °C, the Q_{10} of the respiration is close to 2, but it becomes much less in the high-temperature range from 25 to 32 °C (Yoshida, 1981). When rice seedlings were exposed to different high temperatures (35, 40, and 45 °C) for 48 h, the maximal quantum yield of photosystem II (PSII) photochemistry, the activity of ascorbate peroxidase, and the proteome changes were greater at higher temperature (Han *et al.*, 2009). The higher the temperature, the more protection mechanisms will be involved.

2.3. Leaf emergence

A moderate increase in temperature speeds up leaf emergence, and temperature is a principal environmental determinant of leaf appearance in rice (Gao *et al.*, 1992; Ritchie, 1993). The phyllochron concept, which is defined as

the time interval between the appearances of successive leaf tips (Klepper *et al.*, 1982), is used to predict the appearance of individual leaves, expressed in thermal time, with units of degree days. The leaf appearance rate (LAR) is not constant with time when rice plants are grown at constant temperature (Yin *et al.*, 1996), suggesting an effect of age. The leaf number is linearly related to cumulative thermal units (TU, degree days (°Cd)) from seedling emergence (Yoshida, 1981). The inverse of the slope of this linear relation provides an estimate of the phyllochron. Rice plants can be described by a base temperature below which development stops ($T_{base} \sim 8$ °C), and an optimum temperature ($T_{opt} \sim 25$ °C) at which the development rate is the fastest. Ellis *et al.* (1993) used a quadratic equation to describe the relationship between LAR and temperature and showed that the optimum temperature (T_{opt}) for LAR of cv. IR36 was about 26 °C, at least 2 °C lower than the optimum for phenological development to flowering.

In terms of the temperature summation index the development of one leaf requires about 100 degree days before the initiation of panicle primordia and, about 170 degree days thereafter. Thus, when rice plants are grown at 20 °C, leaves emerge every 5 days (100 degree days/20 °C = 5 days); when grown at 25 °C, they emerge every 4 days before panicle primordial initiation. Since leaf appearance is controlled by temperature near the apical meristem (Ritchie, 1993), the floodwater temperature may play an important role in the fields.

2.4. Tillering

Tillers are branches that develop from the leaf axils at each unelongated node of the main shoot or from other tillers during vegetative growth, growing independently by means of its own adventitious roots. Tillering is a two-stage process: the formation of axillary buds at each leaf axil and its subsequent growth. Yoshida (1973) reported that higher temperatures increased tiller numbers. At 3–5 weeks after sowing, temperature only slightly affected the tillering rate and the relative growth rate, except at the lowest temperature (22 °C) tested. Tiller number per plant determines panicle number which is a key component of grain yield (Yoshida, 1981). To some extent, yield potential of a rice cultivar may be characterized by tillering capacity. But, rice plants with more tillers can show a greater inconsistency in mobilizing assimilates and nutrients among tillers, resulting in variations in grain development and yield among tillers (Yoshida, 1981). There appears to be a synchronism in emergence between main stems and tillers and further, between tillers themselves. High temperatures may affect this synchronism and in the mobilization of assimilates and nutrients among tillers.

2.5. Heading

Physiologically, heading time (days from sowing to heading) can be divided into two developmental stages: vegetative growth time and reproductive growth time. The vegetative growth time can be further divided into the basic vegetative phase and the photoperiod-sensitive phase. As temperature increases, development generally accelerates as a linear function of daily average temperature. This developmental response to temperature has provided the growing degree-day concept and does well to describe rice development as long as temperatures remain within 24–35 °C. Nevertheless, a nonlinear model is needed to describe development when a crop is exposed to high-temperature stress. Even within a daily mean temperature range of 21–30 °C, the number of days to heading is not linearly related to temperature. When temperature drops from 24 to 21 °C, there is a sharp increase in days to heading. For example, the number of days to heading for IR26 increased from 96 days at 24 °C to 134 days at 21 °C (Yoshida, 1981). When the temperature was increased above 24 °C, however, heading time decreased to 91 days at 27 °C and to 86 days at 30 °C. These effects suggest the existence of a *ceiling temperature*. Generally, high temperature accelerates and low temperature delays heading (Ahn and Vergara, 1969; Hosoi and Tamagata, 1973). In contrast, Asakuma and Iwashita (1961) and Azmi (1969) reported that high temperature delayed flowering. A generalized relationship between temperature and length of time required to complete development shows that the existence of a critical low temperature below (normally below 20 °C) which the plant will not progress to anthesis. An intermediate optimum temperature permits the most rapid development. Adverse temperatures above the optimum cause a lengthening of the time required for development. There is no linear relationship between temperature and growth duration, limiting the use of temperature summation (Yoshida, 1981).

2.6. Growth

Depending upon genotype and environment, rice plants take about 3–6 months from germination to harvest. There are two sequential growth stages: vegetative phase from germination to panicle initiation and reproductive phase from panicle initiation to maturity. In its biphasic growth pattern, the first half phase of vegetative growth of rice precedes the second phase of reproductive growth (Yoshida, 1981). Temperature strongly influences the rates at which these phases proceed and is probably one of the reasons for the different crop durations in temperate and tropical environments. The entire growth process from germination to maturity includes many component physiological and biochemical processes. Some processes may be temperature insensitive, others may be linearly dependent on

temperature, and still others may be logarithmically dependent on temperature. On the whole, temperature influences growth rate, duration, and productivity. In the temperature range of 22–31 °C, the growth rate increases linearly. But, higher temperature adversely affects growth and productivity (Yoshida, 1981). Typically, a rice crop requires about 2000–4000 degree days, which corresponds to 80–160 days, depending on the cultivar and location. However, the implicit assumption of the temperature summation concept that growth rate or developmental rate is a linear function of temperature is an oversimplification. Because, (i) a rise in temperature increases the rate at which leaves emerge, (ii) the number of developed leaves on the main culm before heading is fairly constant for a given variety, (iii) the number of days from sowing to heading is fairly constant under a given temperature regime, and (iv) rise in temperature increases the rate of grain filling after flowering in rice plants. The generalized relationship between temperature and length of time required to complete development is curvilinear, indicating that time required for plant development is lengthened below and above optimum temperatures.

2.6.1. Plant height

After transplanting, the aerial growth of rice plants is accelerated linearly from 18 to 33 °C and growth is reported to decrease above or below this temperature range. The plant elongates vigorously until 30 days after transplanting, then slowly ceases to elongate at the heading time. Kondo and Okamura (1931) and Osada *et al.* (1973) also reported that the plant height increased with the rise of temperature within the range of 30–35 °C. Kondo and Okamura (1931) suggested that the optimum temperature for dry-matter production was lower than or equal to that for stem elongation. In a recent study, Oh-e *et al.* (2007) reported that the increase in plant height was steeper under high temperature than under ambient temperature condition.

2.6.2. Tillers and panicles

The optimum temperature for tillering is 25 °C at day and 20 °C at night (Sato, 1972). Tillering increases with rising temperature in the range of 15–33 °C. Chaudhary and Ghildyal (1970) found that temperatures above 33 °C were unfavorable for tillering. Oh-e *et al.* (2007) observed that the number of tillers per square meter during the early growth period was generally larger under high temperature and the maximum tillering stage was earlier than under normal temperature conditions. At maturity, the number of tillers was found to be lower in high-temperature conditions than in ambient conditions inside a temperature gradient chamber (TGC; Oh-e *et al.*, 2007).

Panicle differentiation occurs generally at temperatures between 18 and 30 °C. During tillering stage, the number of panicles will increase if the air

temperature is lower than 20 °C (Yamamoto *et al.*, 1985). After the active-tillering stage, high temperatures decrease the number of panicles, especially at maturity. In addition to the influences of air temperature, the floodwater temperature affects the number of panicles per plant and spikelets per panicle. At the early stage of growth, the growing points of leaves, tillers, and panicles are under water, when rice is cultivated under irrigated or lowland rain-fed conditions. At later stages of plant growth, panicle growth and ripening are influenced more by the air temperature than that of the floodwater.

2.6.3. Panicle dry weight

The optimal temperature for ripening is lower than that for tillering and anthesis. The temperature optimum shifts to relatively lower temperatures as rice grows. The panicle weight is known to decrease under high temperature (Newman *et al.*, 2001; Oh-e *et al.*, 2007; Ziska *et al.*, 1996). Kim *et al.* (1996a,b) reported that the rate of increase in dry matter in the panicle after the heading decreased under high temperature. This could be partly due to the increase in the number of sterile spikelets. The dry weight of panicle will not recover and the assimilation products will accumulate in leaves and culms, even if the subsequent conditions are favorable for panicle development.

2.6.4. Dark respiration

Dark respiration is a key physiological process in growth and maintenance of plants since a portion of most of the growth- and maintenance-dependent activities are respiration dependent. Respiration is considered to be a good indicator of physiological activity (Henderson, 1934). Increased respiration loss could cause the decreases in average grain weight despite the availability of carbohydrates in leaves and culms (Morita *et al.*, 2004). Oh-e *et al.* (2007) observed that the specific dark respiration for the whole plant was low at transplanting, reached the maximum value at the tillering stage, and gradually decreased thereafter. Under high temperatures, rice plants may show high dark respiration at maturity. During ripening period, higher dark respiration rate under high temperatures may be associated with the increase in the amount of substrate for respiration.

2.6.5. Grain filling

High temperatures at flowering and during grain-filling phase reduce yield by causing spikelet sterility and shortening the duration of grain-filling phase (Tian *et al.*, 2007; Xie *et al.*, 2009). For a particular cultivar, the growing degree days required for flowering is relatively the same at different growing temperatures within the temperature range between the base temperature and the optimum temperature. Yoshida and Hara (1977) and Oh-e *et al.* (2007) observed that the rate of grain growth was faster and the grain-filling

period was shorter at higher temperatures. High temperatures above 30 °C are generally not favorable for ripening (Osada *et al.*, 1973). Morita *et al.* (2005) reported that high night temperatures (22/34 °C, day/night) were more harmful to grain weight in rice than high day temperatures (34/22 °C) and control conditions (22/22 °C) at optimum temperature. The final grain weight which is the product of the rate and duration of grain growth is affected by high temperatures which increase growth rate in the early ripening period but reduce the duration of grain growth and ultimately result in decreases in final grain weight. The length of the ripening period is inversely correlated with daily mean temperature and, therefore, grain filling is poor when temperature is above optimum, although a rise in temperature increases the rate of grain filling. The duration of grain filling, defined as the number of days required to reach maximum weight, was found to be 13 days at a mean temperature of 28 °C, and 33 days at 16 °C for cultivar IR20, an *indica* rice. But, the cultivar Fujisaka 5, a *japonica* rice, took a little longer to ripen: 18 days at a mean temperature of 28 °C and 43 days at 16 °C (Oh-e *et al.*, 2007). Since the time of heading and anthesis may vary among panicles and spikelets within the same panicle, the duration of grain filling under field conditions will be much greater. Interestingly, the final grain weights attained at high and low temperatures could be similar or different. Oh-e *et al.* (2007) found that the cultivar IR20 was well adapted to high temperatures during ripening while the final grain weight of cultivar Fujisaka 5 at 28 °C was about 15% less than that at 16 °C, suggesting that certain cultivars may show the detrimental effect of high temperatures.

2.6.6. Grain quality

Grain yield is not the only consideration in the cultivation of rice, and grain dimensions, the appearance in terms of color, texture, and surface abnormalities and milling characteristics are also important factors regulating the popularity and marketability. Owing to high temperatures during the ripening period, abnormal morphology and coloration occur in rice, probably due to reduced enzymatic activity related to grain filling, respiratory consumption of assimilation products and decreased sink activity (Inaba and Sato, 1976; Tsukaguchi and Iida, 2008). The chalkiness is one of the key factors in determining rice quality and price. In Japan, chalky grains are conventionally classified into different categories such as milky white rice, white-core rice, white-belly rice, white-based rice, and white-back rice (Yoshioka *et al.*, 2007). Wakamatsu *et al.* (2007) observed that the incidences of white-back kernel and white-based kernel were high when an average temperature during the 20-day period after heading was 27 °C or higher. Below that temperature, no such incidence was apparent. On cooking, chalkiness disappears and has no effect on taste or aroma. But, it detracts from the appearance and thus decreases market acceptance. Because the husked rice is thicker and the protein content is lower in white-back

kernel than in milky white kernel, the effect of white-back kernel on taste deterioration is presumed to be less than that of milky white kernel. With average temperatures of 26 °C or higher during the 30-day period after heading, the grain weight tended to be lighter in the condition, whereas the whole grain ratio to the total number of grains tended to decrease at 27 °C or at higher temperatures. In general, the temperature suitable for ripening is considered to be 24 °C at which temperature the maximum grain weight is observed (Kobata *et al*., 2004). There may be differences among cultivars in the ratio of imperfect rice incidence, suggesting that the cultivar difference in the pattern and severity of the incidence and the ripening capability at high temperature are genetically controlled.

2.6.7. Grain fissuring

Rice is primarily consumed as an intact grain and therefore production quality is largely measured by head rice yields, which is the mass percentage of rough rice grains that remain as head rice. The broken rice is worth only 50–60% of the value of head rice. Harvesting time should avoid grain fissure formation due to rapid moisture adsorption (Kunze, 1977) and improper drying and storage procedures can also cause grain fissuring that can reduce head rice yield (Daniels *et al*., 1998). From the field and pot experiments to elucidate the effect of meteorological conditions during grain filling on grain fissuring in rice using a total of 13 cultivars, Nagata *et al*. (2004) found that the percentage of fissured grains was closely related with the temperature and solar radiation conditions during the early stage of grain filling. High temperature and long sunshine hours during this period increased the grain fissuring of all cultivars tested although the cultivars are known to differ in their susceptibility to fissuring. They also found that the average daily maximum temperature during 10 days after heading showed the highest correlation with the percentages of fissured grains. High-temperature treatments when given at 6–10 days after flowering, during which the dry weight of spikelets was 14–40% of that at maturity, caused the greatest grain fissuring. Nagata *et al*. (2004) concluded that high temperatures during the early stage of grain filling increased the rice grain fissuring at maturity. The selection of rice cultivars with some variation in maturity to spread pollination over a number of days provides an advantage during the adverse weather conditions at flowering.

2.6.8. Yield

The yield capacity of rice is primarily dependent on both vegetative (number of panicles per unit area) and reproductive (number of spikelets per panicle) phases. The actual yield is realized at flowering and during grain-filling (filled spikelet percentage and weight per grain) phases. Temperature influences rice yield by directly affecting the physiological processes involved in grain production. During the reproductive stage, the spikelet

number per plant increases as the temperature drops. In general, the optimal temperature shifts from high to low as growth advances from the vegetative to the reproductive stages. As early as 1958, Matsushima and Tsunoda reported that the mean optimum temperature for ripening of *japonica* rice in Japan was about 20–22 °C. Although temperature during ripening affects the weight per grain, the 1000-grain weight of a particular cultivar is considered to be almost constant under different environments and cultural practices. However, Murata (1976) observed that the 1000-grain weight of the same variety varied from about 24 g at a mean temperature of 22 °C in the 3-week period after heading to 21 g at a mean temperature of 28 °C in Kyushu, southern Japan. A daily mean temperature as high as 29 °C is not considered to be detrimental to ripening when solar radiation is high in the tropics. Though Murata (1976) observed that the *indica* cultivars are better adapted to high temperatures, while *japonica* varieties require low temperature for better ripening, such observations were not found by Prasad *et al.* (2006). Since the length of ripening is inversely correlated with daily mean temperature, high temperature can seriously impair ripening. Both grain weight and percentage of filled spikelets are affected by high temperatures.

3. Symptoms of High-Temperature Injury in Rice

In an ecosystem, rice plants have to adapt to the prevalent soil and weather conditions. The adaptive mechanisms of plants enable them to tolerate these conditions and reflect the environment in which rice has evolved. The ability of rice plants to tolerate higher temperatures depends on different thermotolerance mechanisms at biochemical and metabolic levels, membrane stability, synthesis of heat shock proteins (Hsp), and photosynthetic activities.

3.1. External symptoms

The high-temperature effects can be at different levels of organization such as biochemical, physiological, morphological, and whole plant systems. When rice plants are exposed to temperatures higher than 35 °C, injuries due to heat occur according to growth stages. In general, white leaf tips, chlorotic bands and blotches, and white bands and specks often develop on the leaves, which are commonly observed in rice plants when grown in a heated glasshouse during winter in the temperate regions (Table 1).

Table 1 Consequence of high temperature on morphological parameters in rice

Morphological parameter	Temperature treatments (°C)	Experimental facility	Association	Impact	Reference
Chlorotic bands and blotches on leaves	>35	GC	Positive	—	Yoshida *et al.* (1981)
Effective tiller number	26–31	TGC	Positive	20–40%	Kim *et al.* (1996b)
Growth duration	25/18 to 34/27	Sunlit CEC	Negative	By 10 days	Baker and Allen (1993b)
Growth duration	29/21 to 37/29	Sunlit CEC	Negative	−12%	Manalo *et al.* (1994)
Growth duration	Ambient +5	TGC	Negative	6–8 days	Prasad *et al.* (2006)
Growth duration	30.4/21.2 to 39.7/22.1	TGC	Negative	−2%	Oh-e *et al.* (2007)
Leaf area	40/33 and 28/21	Sunlit CEC	Negative	−62.5%	Baker *et al.* (1992)
Leaf area	Ambient +4	OTC	Positive	30%	Lin *et al.* (1997)
Leaf area	28 and 32 night temperature	Greenhouse	Positive	+200%	Mohammed and Tarpley (2009b)
Leaf size	40/33 and 28/21	Sunlit CEC	Negative	–	Baker *et al.* (1992)
Leaves curled severely and leaf tips dry	40 and 45	GC	Positive	–	Han *et al.* (2009)
Leaves per plant	28 and 32 night temperature	Greenhouse	Negative	−10%	Mohammed and Tarpley (2009b)
Number of main stem leaves	25/18 to 34/27	Sunlit CEC	Negative	–	Baker and Allen (1993b)
Panicle emergence	28 and 32 night temperature	Greenhouse	Negative	2 days earlier	Mohammed and Tarpley (2009b)
Phylocron interval	29/21 to 37/29	Sunlit CEC	Negative	−15%	Manalo *et al.* (1994)
Plant height	29/21–37/29	Sunlit CEC	Negative	−10%	Manalo *et al.* (1994)
Plant height	30.4/21.2 to 39.7/22.1	TGC	Negative	−3%	Oh-e *et al.* (2007)
Plant height	28 and 32 night temperature	Greenhouse	No effect	–	Mohammed and Tarpley (2009b)
Plant height	26–31	TGC	No effect	–	Kim *et al.* (1996b)

(*Continued*)

Table 1 (*Continued*)

Morphological parameter	Temperature treatments (°C)	Experimental facility	Association	Impact	Reference
Plant height	20/25, 25/25, and 45/25	Control chambers	Negative	−73%	Yoshida *et al.* (1981)
Rate of appearance of new leaf	29/21 to 37/29	Sunlit CEC	Positive	5–30%	Manalo *et al.* (1994)
Tiller number	15–33	GC	Negative	–	Nishiyama (1976)
Tiller number	29/21 to 37/29	Sunlit CEC	Negative	−25%	Manalo *et al.* (1994)
Tiller number	30.4/21.2 to 39.7/22.1	TGC	Negative	−15%	Oh-e *et al.* (2007)
Tiller number	40/33 to 28/21	Sunlit CEC	Positive	30%	Baker *et al.* (1992)
Tiller number	28 and 32 night temperature	Greenhouse	No effect	–	Mohammed and Tarpley (2009b)
Tiller number	26–31	TGC	Positive	30–75%	Kim *et al.* (1996b)
Tiller number/plant	30/25, 35/25, and 45/25	Control chambers	Negative	−77%	Yoshida *et al.* (1981)
Time to panicle emergence	29/21–37/29	Sunlit CEC	Negative	−9%	Manalo *et al.* (1994)
Time to panicle emergence	37/29 and 29/21	Sunlit CEC	Negative	−9%	Ziska *et al.* (1996)
White leaf tip	>35	GC	Positive	–	Yoshida *et al.* (1981)

GC, growth chamber; TGC, temperature gradient chamber; CEC, control environmental chamber; OTC, open-top chamber.

3.2. Ultrastructural changes

Under high-temperature stress conditions, there is a tendency for reduced cell size, closure of stomata and curtailed water loss (usually not observed in high light conditions, until there has been a temperature more than 35 °C), increased stomatal and trichomatous densities, and greater xylem vessel numbers of both root and shoot (Banon *et al.*, 2004). Lysis of cytoplasm, accumulation of electron-dense granules in the cytoplasm, distension in the endoplasmic reticulum membranes, enhanced association of ribosomes with the endoplasmic reticulum, reduction in the number of mitochondrial cristae, and disorganization of cell wall fibrillar material are also observed due to high-temperature stress (Pareek *et al.*, 1997). High temperature is found to enhance discontinuity in the plasma membrane with loose association of osmiophilic granules.

In the flag leaves of two rice lines (a thermosensitive line 4628 and a thermo-tolerant line 996), Zhang *et al.* (2009) characterized the microscopic and ultrastructural characteristics of mesophyll cells under high-temperature stress (37 °C during 8:00–17:00 h and 30 °C during 17:00–8:00 h) using an optical and a transmission electron microscopy. High-temperature stress led to different responses; thermo-resistant line 996 showed tightly arranged mesophyll cells in flag leaves, fully developed vascular bundles, and some closed stomata, whereas the line 4628 suffered from injury because of undeveloped vascular bundles, loosely arranged mesophyll cells, and opened stomata. They found that the mesophyll cells in flag leaves of the line 4628 were severely damaged under high-temperature stress conditions. The chloroplast envelope became blurred, the grana thylakoid layer was arranged loosely and irregularly, the stroma layer disappeared, many osmiophilic granules appeared within the chloroplast, the outer membrane of mitochondria and the nucleus disintegrated and became blurred, the nucleolus disappeared, and much fibrillar–granular materials appeared within the nucleus. In contrast, the mesophyll cells in flag leaves of the line 996 maintained an intact ultrastructure under the high-temperature stress. Zhang *et al.* (2009) suggested that the primary response of rice plants to high temperature was the ultrastructural modification of the cell membrane system, which could be used as an index to evaluate the crop heat tolerance. Evidently, high-temperature stress considerably affects anatomical structures not only at the tissue and cellular levels but also at the subcellular level.

3.3. Phenological changes

High-temperature stress is a major factor affecting the rate of plant development, which is considered to increase to a certain limit and then decrease afterward (Hall, 1992; Howarth, 2005; Marcum, 1998). The succession of

rice developmental stages (phenology) depends on air and water temperature and on photoperiod (day-length). Thus, the changes in phenology in response to heat stress can reflect the interactions between stress environment and plants. The different phenological events differ in their sensitivity to high temperature, depending on species and genotypes which show inter- and intraspecific variations (Howarth, 2005; Wollenweber *et al.*, 2003). It is unknown whether damaging effects of heat episodes occurring at different developmental stages are cumulative (Wollenweber *et al.*, 2003). All vegetative and reproductive stages are affected by heat stress to some extent: high day temperature can damage leaf gas exchange properties during the vegetative stage and even a short period of heat stress can cause significant increases in the abortion of floral buds and opened flowers during the reproductive stage (Guilioni *et al.*, 1997). Often, the impairment of pollen and anther development by elevated temperatures is an important factor contributing to decreased fruit set in many crops at moderate to high temperatures (Peet *et al.*, 1998; Sato *et al.*, 2006). Since rice plants can tolerate only narrow temperature ranges, especially during the flowering phase, fertilization and seed production are damaged, resulting in reduced yield (Porter, 2005). Earlier heading is advantageous for the retention of more green leaves at anthesis under high-temperature conditions, leading to a smaller reduction in yield later (Tewolde *et al.*, 2006).

3.4. Physiological changes

3.4.1. Role of water

Water plays a vital role in all physiological activities since many metabolic processes such as enzymatic reactions, transportation and accumulation of ions occur in cytosol of living tissues. Even in seeds, the water compartment correlates with the organic properties of macromolecular structures associated with development. High temperature affects the physical status of water in plant cells that reflect cellular activity. The grains of rice plants grown at 30 °C had free water for shorter period (22 days after flowering) than those grown at 20 °C (28 days after flowering; Funaba *et al.*, 2006). Thereafter, they found grains having only loosely bound water and bound water. The formation of chalky grains through loose packing of amyloplasts is generally due to high-temperature stress. In an investigation on the changes in water distribution in the developing caryopses by high-temperature stress, Ishimaru *et al.* (2009) observed lower-water content around the center of the endosperm from the magnetic resonance images of the early stage rice caryopses in the high-temperature condition and disorganized development of amyloplasts by the scanning electron microscopy.

3.4.2. Accretion of osmolytes

Under abiotic stresses, like salinity, water deficit, and extreme temperature, rice plants accumulate certain organic compounds of low molecular mass, generally referred as compatible osmolytes. The osmolytes are of diverse nature: sugars, sugar alcohols (polyols), proline, tertiary and quaternary compounds, and glycinebetaine are some of them. The accumulation of osmolytes in plant cells can result in a decrease of the cell osmotic potential and in maintenance of water absorption and cell turgor pressure, all of which contribute to sustain processes such as stomatal opening, photosynthesis, and growth. Since the heat stress is highly complex, the functional significance of osmolyte accumulation has not been fully appreciated (Wahid *et al.*, 2007).

3.4.3. Chlorophyll fluorescence

In the chlorophyll molecules of a leaf, light energy can drive photosynthesis, be dissipated as heat, or reemitted as light, that is, chlorophyll fluorescece, and these three processes occur in competition. By measuring the yield of chlorophyll fluorescence, changes in the efficiency of photochemistry and heat dissipation can be obtained. Yamada *et al.* (1996) suggested that the physiological parameters such as chlorophyll fluorescence, the ratio of variable fluorescence to maximum fluorescence (F_v/F_m), and the base fluorescence (F_0) correlate with heat tolerance. The maximal quantum yield of PSII photochemistry (F_v/F_m) is an important parameter for the PSII activity and any decrease in F_v/F_m indicates the loss of PSII activity. Han *et al.* (2009) found that F_v/F_m value was 0.836 at 26 °C, but decreased slightly (0.817) at 35 °C, and significantly to 0.782 under 40 °C and to 0.62 under 45 °C, indicating the inhibition of PSII activity under high-temperature stress condition.

Many physiological changes like decreases in photosynthesis, water-use efficiency, nutrient-use efficiency, an increase in respiration rate, membrane injury, evapotranspiration, and so on have been observed by many researchers under different high-temperature stress experimental conditions and are discussed in the subsequent sections of this review.

4. High-Temperature Injury and Rice Crop Production

As the most common tropical food cereal, rice is generally considered to be adapted to high-temperature regions. Nevertheless, optimum temperatures exist for each growth stage, and that temperatures exceeding the optimum often occur under field conditions (Owen, 1971). As these plants

cannot physically move away from high-temperature conditions, the ability to respond and ultimately to adapt to high-temperature stress is very essential for their survival, growth, and productivity. There are also cultivars with varying durations of lifecycle: some are early maturing, while others are late maturing.

4.1. Growth-stage-dependent responses

The growth duration of a rice crop varies from 3 to 8 months depending on the cultivars and environmental conditions. The development from germination to maturity has a series of discrete periods, each identified by the changes in structure, size, or mass of specific organs. Under tropical conditions, a typical 120-day cultivar has about 60 days of vegetative growth and about 30 days each of reproductive and ripening growth (Yoshida, 1981). The leaf weight increases up to flowering and then decreases due to drying and death of lower leaves. Likewise, the dry weight of leaf sheath and culm increases up to flowering, followed by a decline due to translocation of accumulated plant reserves to panicles. The vegetative phase is divided into two subphases: (i) the active-vegetative phase that lasts to maximum tillering and is accompanied by a rapid increase in plant height and tiller number and dry-matter production and (ii) vegetative-lag phase continues up to panicle initiation. During the vegetative-lag phase, maximum tillering, internode elongation, and panicle initiation occur almost simultaneously in cultivars of 105–120 days duration and successively later in cultivars of more than 140 days duration. The physiological growth stage is generally indicated by the number of fully developed leaves on the main stem (De Datta, 1981). The reproductive phase, which is characterized by the culm elongation, emergence of the flag leaf, booting, heading, and filling of the spikelets begins just before or after the maximum tillering. Temperature affects the growth duration of the rice crop to a great extent. When rice is exposed to high air temperatures during the vegetative stage, individual plant height, tiller number, and dry weight may be considerably reduced.

Temperatures above 35 °C cause different types of heat injury to rice crop, depending on the cultivar and growth stage (Yoshida *et al.*, 1981). There are reports that the total dry weight of cv. IR747B2-6 at 35/25 °C was only one-sixth of that at 30/25 °C. In 2 days at 45/25 °C, leaves became discolored and desiccated, gradually dried from the tip to the base, and died 9 days later (Yoshida *et al.*, 1981). Rice is basically a photoperiod-sensitive, short-day plant. But the development of day-neutral (photo-insensitive) cultivars has led to introduction of many cultivars which mature within a fixed duration and can be planted any time during the year in the tropics. Even in the case of photo-insensitive cv. IR26, temperatures above 26 °C were found to decrease the number of days to heading. When

characterizing the effects of [CO_2] (330 and 660 μmol mol^{-1}) and temperature regimes (29/21, 33/25, and 37/29 °C, day/night temperature) on growth of three lowland (cv. IR28, IR36, and IR64) and three upland (cv. ITA186, Moroberekan, and Salumpikit) rice cultivars under the controlled-environment conditions, Manalo *et al.* (1994) found that at a [CO_2] of 330 μmol mol^{-1}, most cultivars grew best at 33/25 °C. Doubling [CO_2] increased plant height by 17% at 29/21 °C and by 7% at 33/25 °C, but reduced plant height by 3% at 37/29 °C. Increasing temperature from 29/21 to 37/29 °C reduced tiller number by 10% but doubling [CO_2] more than offset this effect. Tiller number was 66% greater in the high [CO_2], and high-temperature treatment than in the low [CO_2], and low-temperature treatment at 45 days after sowing. In the lowland cultivars, the combination of higher [CO_2] and higher temperature doubly shortened the vegetative and reproductive phases, while at 29/21 °C, increased [CO_2] delayed onset of the reproductive phase. Interestingly, flowering of cv. ITA186 was not affected by [CO_2]. For lowland cultivars, the total dry weight was inversely related to high temperature. These results suggest that there are significant cultivar differences in responses to temperature and these differences may provide options to minimize adverse effects of future climate changes by selecting and breeding of suitable cultivars for different regions.

4.1.1. Seedling stage

The optimum temperature for germination is between 30 and 35 °C, and under suitable conditions, the seed absorbs water to about 25% of its dry weight. The first indication of germination is detectable after about 2 days. When the growing tips of vegetative parts are under floodwater or soil, its temperature greatly affects the growth and development. High-temperature stress can do harm to germination and seedling emergence and even lead to death if it takes place during the seedling stage. The long-term effects of high-temperature stress may include delayed germination or loss of vigor, leading to reduced emergence and seedling establishment. Several quantitative traits such as seed imbibition rate, germination rate, germination index, shoot length, root length, and seed vigor are associated with seed germination ability at different germination stages. Cao and Zhao (2008) suggested that brassinolide, a recently recognized type of plant growth regulator, plays an important role in protection of rice seedlings from heat stress. After seedling emergence, the root structures in young seedlings show higher weight proportions than shoot and hence, soil temperature also affects their growth and development. Hence, there is a strong need to investigate the specific seed germination traits and seedling growth and development as influenced by high air, water, or soil temperatures.

4.1.2. Flowering

Endogenous and environmental signals determine the transition from vegetative to reproductive growth and two major environmental factors that influence this transition are photoperiod and temperature. The reproductive growth generally begins just before or after the maximum tillering stage and is characterized by culm elongation, emergence of the flag leaf, booting, and heading and filling of the spikelets (Yoshida, 1981). The panicle, composed of a base axis, primary and secondary branches, rudimentary glumes, and spikelets, extends upward inside the flag leaf sheath, and booting (swelling of the flag leaf sheath) occurs in the later part of panicle development, followed by emergence of the panicle out of the flag sheath (heading). Each spikelet contains a single hermaphrodite flower, borne on a short pedicel, which is enlarged at the top with two oblique sides. The opening of spikelet begins either on the day of panicle emergence, more usually on the second day. In most plants, gametogenesis (8–9 days before anthesis) and fertilization (1–3 days after anthesis) are very sensitive reproductive phases to high temperature (Foolad, 2005). Both male and female gametophytes are sensitive, with sensitivity response varying with genotype, and ovules being less heat sensitive than pollen (Peet *et al.*, 1998). Anthesis is the most sensitive stage of rice to high temperatures (Yoshida *et al.*, 1981) and the heat-sensitive processes of anthesis are anther dehiscence, pollination, pollen germination, and to a lesser extent pollen tube growth, which is completed within 45 min of the opening of a rice spikelet (Ekanayake *et al.*, 1989). Fertilization is completed within 1.5–4 h (Cho, 1956).

Effects of high temperature on floral characteristics are given in Table 2. The weather conditions, particularly air temperature, affect the onset of flowering. The optimum temperature for blooming is about 30 °C; flower opening is most prolific between 9 and 12 a.m. in the tropics, with spikelets remain open for 30–90 min. Anthesis takes place either immediately before or simultaneously with spikelet opening. In rice, the reproductive processes that occur within 1 h after anthesis—dehiscence of the anther, shedding of pollen, germination of pollen grains on stigma, and elongation of pollen tubes are more sensitive to high temperatures and are disrupted at day temperatures above 33 °C (Satake and Yoshida, 1978). High temperatures just before or during anthesis are injurious, resulting in lower seed set (Prasad *et al.*, 2006). Sterility is fairly common, varying from a few empty spikelets to almost complete sterility in rice. Unfavorable weather, particularly high temperature may result in a lack of fertilization of spikelets. The most severe effect of high temperature during reproductive growth is induction of sterility (Satake and Yoshida, 1978). High-temperature stress at the heading stage can cause spikelet sterility, resulting in yield loss (Matsui *et al.*, 1997a). In the traditional cropping patterns, the period of rice cultivation is not preferred under temperatures that cause sterility. But the

Table 2 Floral characteristics affected by high temperature

Parameter	Experimental facility	Temperature treatment (°C)	Impact	Association	References
Anther dehiscence	Phytotron	29, 35, 38, and 40	Positive	–	Satake and Yoshida (1978)
Anther dehiscence	–	–	Positive	–	Zheng and Mackill (1982)
Diameter of the pollen grains	Sunlit phytotron	34–39	Negative	−8%	Matsui *et al.* (2000, 2001)
Duration to flower	SPAR	28/18, 34/24, and 40/30	Negative	17 days earlier	Gesch *et al.* (2003)
Duration to flower	OTC	25.6 and 29.5	Negative	5 days earlier	Lin *et al.* (1997)
Duration to flower	TGC	30.4/21.2 and 39.7/22.1	Negative	−3%	Oh-e *et al.* (2007)
Percentage of dehised thecca	Sunlit phytotron	34–39	Negative	10–100%	Matsui *et al.* (2000, 2001)
Pollen fertility	Phytotron	32 and 39	Negative	65%	Tang *et al.* (2008)
Pollen germination	Artificial media	12 and 43	Negative	–	Enomoto *et al.* (1956)
Pollen germination	–	28 and >35	Negative	−12%	Li *et al.* (2002)
Pollen germination	Glasshouse	38/27 and 29/21	Negative	40–90%	Mackill *et al.* (1982)
Pollen germination	Greenhouse	25, 36.5, 38, and 39.5	Negative	−20%	Matsui *et al.* (1997a,b)
Pollen germination	Greenhouse	28 and 32 night temperature	Negative	−20%	Mohammed and Tarpley (2009b)
Pollen germination	Phytotron	32 and 39	Negative	−75%	Tang *et al.* (2008)
Pollen germination	–	–	Negative	–	Xu *et al.* (2001)

(*Continued*)

Table 2 (*Continued*)

Parameter	Experimental facility	Temperature treatment (°C)	Impact	Association	References
Pollen germination	–	43 °C for 7 min	Negative	−100%	Yoshida (1981)
Pollen production	–	28 and >35	Negative	−30%	Li *et al.* (2002)
Pollen production	Sunlit phytotron	34–39	Negative	–	Matsui *et al.* (2000, 2001)
Pollen production	TGC	Ambient +5	Negative	−52.6%	Prasad *et al.* (2006)
Pollen production	–	–	Negative	–	Xu *et al.* (2001)
Pollen shed on the stigmas	–	28 and >35	Negative	−13%	Li *et al.* (2002)
Pollen shed on the stigmas	Glasshouse	38/27 and 29/21	Negative	−5–50%	Mackill *et al.* (1982)
Pollen shed on the stigmas	Greenhouse	25, 36.5, 38, and 39.5	Negative	−30%	Matsui *et al.* (1997a,b)
Pollen shed on the stigmas	Sunlit phytotron	34–39	Negative	−5–200 grains	Matsui *et al.* (2000, 2001)
Pollen shed on the stigmas	Growth chamber	35	Negative	–	Morokama and Yasuda (2004)
Pollen shed on the stigmas	TGC	Ambient +5	Negative	−42.80%	Prasad *et al.* (2006)
Pollen shed on the stigmas	Phytotron	29, 35, 38, and 40	Negative	−10 to −70%	Satake and Yoshida (1978)
Pollen shed on the stigmas	–	–	Negative	–	Xu *et al.* (2001)

Parameter	Facility	Temperature	Effect	Change	Reference
Pollen shed on the stigmas	Phytotron	29, 35, 38, and 40	Negative	−10 to −70%	Yoshida (1981)
Pollen sterility	Greenhouse	40/21 and 30/21	Positive	5.9–28.4%	Cao *et al*. (2009)
Polllen viability	TGC	Ambient +5	Negative	−16.40%	Prasad *et al*. (2006)
Spikelet fertilization rate	Greenhouse	40/21 and 30/21	Negative	−2–5%	Cao *et al*. (2009)
Spikelet tissue temperature	Growth cabinets	29.6–36.2	Negative	−3.7–4.7%	Jagadish *et al*. (2007)
Time of flowering in the day	Growth cabinets	29.6–36.2	Negative	0.5–1.5 h earlier	Jagadish *et al*. (2008)
Time of flowering in the day	Field	44/28	Negative	7–9 a.m.	Nishiyama and Blanco (1980)
Time of flowering in the day	–	>35	Negative	3 h earlier	Yoshida *et al*. (1981)
White spikelets	–	38/27	Positive	38%	Yoshida *et al*. (1981)

GC, growth chamber; TGC, temperature gradient chamber; CEC, control environmental chamber; OTC, open-top chamber.

intensified cropping patterns that include altered planting dates or planting in different seasons can expose rice plants to adverse temperatures that may occur at critical growth stages (Coffman, 1977). At present, the development of irrigation systems to intensify rice cultivation, especially in continents of South and Southeast Asia and West Africa, allows dry-season cropping of rice in hot months. The predicted higher temperature due to climate change will decrease yield in dry seasons but may not have large effect in wet seasons.

Under the phytotron condition, the combining ability of six rice lines for high-temperature tolerance was characterized at anthesis by Mackill *et al.* (1982). Control plants were grown under a 29/21 °C temperature regime and the treated plants were subjected to 38/27 °C for 10 days during anthesis. Heat tolerance index (the percentage of filled grains of the treated plants divided by that of the control plants) showed highly significant, general and specific combining ability effects. The tolerant lines such as N22, IR2006, and IET4658 were found to have general combining ability effects of 6.80, 4.08, and 3.02, respectively, while the susceptible lines such as IR28, IR1561, and IR52 had −3.40, −4.92, and −5.58, respectively.

In the early ontogeny of the anther, hypodermal archesporial initials divide periclinally to form primary parietal cells and primary sporogenous cells. The anther wall is formed by anticlinal and periclinal divisions of the primary parietal cells as well as surrounding primary sporogenous cells. There exists a relationship between morphological characteristics of anthers and fertility in *japonica* rice cultivars subjected to high temperature (37.5/26 °C day/night) at flowering (Matsui and Omasa, 2002). The number of cell layers that separate the anther locule from the lacuna that formed between the septum and the stomium is negatively correlated with percentage fertility. The cell layers consist of the remaining septum and degraded tapetum, and serve to keep the adjacent two locules closed. Therefore, the anther dehiscence requires the rupture of the cell layers. Tight closure of these locules by the cell layers may delay locule opening and decrease fertility at high temperatures. In a study on the relationship between the length of dehiscence at the basal part of thecae and the viability of pollination in 18 cultivars of rice (Matsui *et al.*, 2005), plants were subjected to a hot and humid condition (37/25 °C, day/night, and >90% relative humidity (RH)) for 3 days at flowering. Control plants were left under ambient conditions in a semicylindrical house covered with cheesecloth (30% shading; temperature range 24–35 °C). The length of basal dehiscence of thecae was found to be strongly correlated with the percentage of florets having more than 80 pollen grains on the stigma under ambient condition ($r = 0.72$, $P < 0.001$). The percentage of florets was more than 20 pollen grains on the stigma under hot conditions ($r = 0.93$, $P < 0.001$). These results indicate that the length of basal dehiscence correlated with pollination or viability under both conditions. The length of the basal dehiscence

was found to be shorter in the non-*japonica*-type cultivars than in many of the *japonica*-type cultivars under both conditions. Hence, low pollination viability in the non-*japonica*-type cultivars is associated with their small basal dehiscence on the theca, and the length of basal dehiscence can be used as a selection marker of high-temperature tolerance (Matsui *et al.*, 2005).

Rice cultivars show decreases in pollen activity, pollen germination, and floret fertility at high temperatures, with tolerant cultivars showing a slower rate of decrease than susceptible cultivars (Tang *et al.*, 2008). In addition, high temperature causes decreases in the contents of indole-3-acetic acid, gibberellic acids, free proline, and soluble proteins, but increases in abscisic acid. There are variations in the severity of these changes in rice cultivars, which indicate that rice floral development is sensitive to heat stress. The rice cultivars may show variations in their adaptability to heat avoidance by changing characteristics of flowering: the length of flowering period, weakening of apical grain superiority, rate of glume opening, the daily number of spikelet flowering, changes in flowering clock, and the rate of grain setting. All of the physiological and morphological features are altered under high-temperature stress (Tao *et al.*, 2008).

High or low temperatures at meiosis stage affect the seed-setting rates. With the increase of temperature and its duration, the seed-setting rate decreases gradually. The relationship between daily relative seed-setting rate and temperature can be fitted with a quadratic equation. However, total effect of high temperature during meiosis stage can be described by the products of these daily relative seed-setting rates (Shi *et al.*, 2008). Heat stress during meiosis influences the development of anther and pollen grains, significantly reducing anther dehiscence and pollen fertility rate and yield components such as number of spikelet per panicle, seed-setting rate, 1000-grain weight, and grain yield (Cao *et al.*, 2008). Among various physiological parameters that are associated with heat stress, decreases in oxidation activity in roots and the RNA content in young panicles and increases in the malondialdehyde content in leaves and the ethylene evolution rate in young panicles suggest that heat tolerance is due to high activity of roots, strong antioxidative defense system, high RNA content, lower ethylene synthesis, and low-malondialdehyde content during meiosis (Cao *et al.*, 2008).

Even high temperatures and high UV-B radiation (18.1 kJ/m^2 day) applied experimentally from 2 weeks before heading increased sterility and decreased the size of unhulled grain and anther length. At the heading stage, sterility was increased and anther length and pollen production were decreased (Inaba, 2005). High temperature and strong UV-B radiation will have synergistic effect, causing poor growth and injurious effects on sterility and pollen formation. There is a strong need to separate tolerance from avoidance and the most tolerant cultivar found to date, cv. N22, is agronomically poor. Tolerance and avoidance of high temperature at anthesis

are potentially useful traits for breeding programs of rice for increasing temperature projected in future climate (IRRI, 2007).

4.2. Yield and its components

Different yield components such as the number of panicles per unit land area, the number of spikelets per panicle, the percentage of filled spikelets, and 1000-grain weight determine grain yield. The number of panicles is closely associated with grain yield, but there is often a negative correlation between the number of panicles per unit land area and spikelets per panicle and between spikelets per unit land area and filled-grain percentage or 1000-grain weight (Yoshida, 1983). High temperature (40/33/37 °C, daytime dry bulb air temperature/nighttime dry bulb air temperature/paddy water temperatures) during stem elongation led to death of rice plants while CO_2 enrichment (660 μmol CO_2 mol^{-1} air) helped plants to survive, but with sterile panicles (Baker *et al.*, 1992). The relative importance of different components of grain yield varies with the location, season, crop developmental duration, and land situation. Each component differs not only with respect to the growth stage at which it is determined but also in its relative contribution to grain yield. The component of spikelets per square meter contributes more to yield, followed by the filled spikelet percentage and grain weight together. The number of spikelets per unit area is the product of number of panicles, depending on the total number of tillers formed and the percentage of productive tillers and spikelets per panicle. High solar radiation combined with relatively low temperature is favorable for the production of spikelets (Venkateswarlu and Visperas, 1987). The number of spikelets produced per unit dry weight, especially between panicle initiation and flowering, or nitrogen absorbed is higher in cool regions than in warm regions (Yoshida, 1983).

As the product of spikelets per panicle and percentage of filled spikelets, the number of filled grains is determined by the source capacity and translocation efficiency. Temperature affects the filled-grain percentage by controlling the capacity of grains to accept carbohydrates and the length of the ripening period, which is inversely correlated with the mean daily temperature (Yoshida, 1983; Table 3). Negative correlation existed between grain yield and mean temperature during the 30 days preceding anthesis (Islam and Morison, 1992). Grain yield and the mean air temperature (27–32 °C) for 20 days after heading time showed an upward convexity and grain yield declined steeply when the mean temperature exceeded 28 °C (Oh-e *et al.*, 2007). In day/night temperature above 28/21 °C, grain yields decline by an average of approximately 10% per 1 °C (Baker and Allen, 1993b). Some of the southern U.S. rice cultivars may be more sensitive to high-temperature stresses during reproductive development than Asian cultivars (Baker, 2004). The daytime temperatures at or above 40–41 °C resulted in zero grain yield, and the upper daytime air

Table 3 Changes in yield and its components under high-temperature conditions

Parameter	Experimental facility	Temperature treatments (°C)	Association	Impact	Reference
Assimilate supply to grain	Plastic film	23 and 29	Negative	–	Kobata *et al.* (2004)
Assimilate supply to grain	–	25/20 and 35/30	Negative	−30%	Sato and Inaba (1976b)
Assimilate supply to grain	Growth chamber and glasshouse	35/30 and 28/23	Negative	−17%	Ito *et al.* (2009)
Filled grain (%)	Field, polyester sheets	Ambient +4	Negative	−28%	Kobata and Uemuki (2004)
Filled grain (%)	Glasshouse	38/27 and 29/21	Negative	−15%	Mackill *et al.* (1982)
Filled grain (%)	Sunlit phytotron	34–40	Negative	−25%	Matsui *et al.* (2001)
Filled grain (%)	OTC	25.6 and 29.5	Negative	−8%	Lin *et al.* (1997)
Filled grain (%)	Greenhouse	25, 36.5, 38, and 39.5	Negative	−50%	Matsui *et al.* (1997a,b)
Filled grain (%)	TGC	26–31	Negative	−20 to −75%	Kim *et al.* (1996b)
Filled grain per panicle	Sunlit chambers	34/27 and 28/21	Negative	−55%	Baker *et al.* (1992)
Filled grain per panicle	TGC	Ambient +5	Negative	−57%	Prasad *et al.* (2006)
Filled spikelets	Sunlit CEC	37/29 and 29/21	Negative	−57 to −88%	Ziska *et al.* (1996)
Grain growth rate	Plastic film	23 and 29	Positive	30–40%	Kobata *et al.* (2004)
Grain size	Sunlit glasshouse	24/19–39/34	Negative	–	Tashiro and Wardlaw (1991a)
Grain size	Greenhouse	40/21 and 30/21	Negative	−3.5%	Cao *et al.* (2009)
Grain weight	Field	23 and 30	Negative	−1 to −3%	Nagato *et al.* (1966)

(*Continued*)

Table 3 (*Continued*)

Parameter	Experimental facility	Temperature treatments (°C)	Association	Impact	Reference
Grain weight	Sunlit glasshouse	24/19–39/34	Negative	−45%	Tashiro and Wardlaw (1991a)
Grain weight	Sunlit chambers	40/33 and 28/21	Negative	−7%	Baker *et al.* (1992)
Grain weight	Field chambers	Ambient +4	Negative	−5%	Kobata and Uemuki (2004)
Grain weight	–	34/22 and 22/22	Negative	−7 to 11%	Morita *et al.* (2004)
Grain weight	Growth chamber	35/24 and 24/18	No effect	–	Counce *et al.* (2005)
Grain weight	–	20–29	Negative	−22%	Wakamatsu *et al.* (2007)
Grain weight	Sunlit chambers	33/27 and 26/20	Negative	−10%	Ishimaru *et al.* (2009)
Grain weight	–	35/30	Negative	−20%	Sato *et al.* (1973)
Grain weight	TGC	30.4/21.2 to 9.7/22.1	Negative	−4%	Oh-e *et al.* (2007)
Grain weight	Control chambers	33/28 or 25/20	Negative	−3 to −5.8%	Yamakawa *et al.* (2007)
Grain weight	Glasshouse	22/34, 34/22, and 22/22	Negative	−14%	Morita *et al.* (2005)
Grain weight	Glasshouse	24/19 to 39/34	Negative	−87%	Tashiro and Wardlaw (1991a)
Grain weight	–	35/30	Negative	−20%	Sato *et al.* (1973)
Grain weight	TGC	26–31	Negative	−8 to −15%	Kim *et al.* (1996b)
Grain weight panicle^{-1}	TGC	Ambient +5	Negative	−48%	Prasad *et al.* (2006)

Harvest index	Sunlit chambers	40/33 and 28/21	Negative	−34%	Baker *et al.* (1992)
Harvest index	TGC	Ambient +5	Negative	−62%	Prasad *et al.* 2006
Harvest index	TGC	26–31	Negative	−40 to −80%	Kim *et al.* (1996b)
Immature grains	–	–	Positive	–	Morita (2008)
Pnicles, no. m^{-2}	TGC	30.4/21.2 to 39.7/22.1	Negative	−7%	Oh-e *et al.* (2007)
Number of effective tillers	Sunlit chambers	40/33 and 28/21	Negative	−80%	Baker *et al.* (1992)
Number of effective tillers	TGC	30.4/21.2 to 39.7/22.1	Negative	−15%	Oh-e *et al.* (2007)
Panicle biomass	Sunlit chambers	40/33 and 28/21	Negative	−100%	Baker *et al.* (1992)
Panicle biomass	OTC	25.6 and 29.5	Negative	−15%	Lin *et al.* (1997)
Panicle biomass	Greenhouse	31/26 and 40/32	Negative	0 to −30%	Zakaria *et al.* (2002)
Panicles, no. $plant^{-1}$	Sunlit chambers	40/33 and 28/21	Negative	−100%	Baker *et al.* (1992)
Panicles, no. $plant^{-1}$	Greenhouse	40/21 and 30/21	No effect	–	Cao *et al.* (2009)
Panicles, no. $plant^{-1}$	Greenhouse	28 and 32 night temperature	No effect	–	Mohammed & Tarpley (2009b)
Panicles, no. m^{-2}	TGC	26–31	Negative	−13 to −20%	Kim *et al.* (1996b)
Panicle weight, g m^{-2}	OTC	25.6 and 29.5	Negative	−15%	Lin *et al.* (1997)
Plant biomass	Sunlit chambers	34/27 and 28/21	Negative	−77%	Baker *et al.* (1992)
Plant biomass	Sunlit chambers	29/21 to 37/29	Negative	−22%	Manalo *et al.* (1994)
Plant biomass	TGC	30.4/21.2 to 39.7/22.1	Negative	−16%	Oh-e *et al.* (2007)
Plant biomass	Growth chambers	19/16, 25/19, 30/24, and 37/31	Negative	−20%	Nagai and Makino (2009)
Ripened grains (%)	TGC		Negative	−33%	Oh-e *et al.* (2007)

(*Continued*)

Table 3 (*Continued*)

Parameter	Experimental facility	Temperature treatments (°C)	Association	Impact	Reference
		30.4/21.2 to 39.7/ 22.1			
Root biomass	Greenhouse	28 and 32 night temperature	Positive	30%	Mhammed and Tarpley (2009b)
Root biomass	Glasshouse	28/23, 35/33, and 38/26	Negative	−17%	Ito *et al.* (2009)
Root biomass	TGC	26–31	Positive	30–70%	Kim *et al.* (1996b)
Root biomass	Control chambers	30/25, 35/25, and 45/25	Negative	−98%	Yoshida *et al.* (1981)
Root dry weight/ total dry weight	Control chambers	30/25, 35/25, and 45/25	Positive	14%	Yoshida *et al.* (1981)
Root dry weight/ total dry weight	TGC	26–31	Positive	25–51%	Kim *et al.* (1996b)
Seed-setting rate	Artificial climate incubators	31, 33, 35, 37, 39, and 41	Negative	−2 to −25%	Shi *et al.* (2008)
Seed-setting rate	Phytotron	32 and 39	Negative	−50%	Tang *et al.* (2008)
Seed-setting rate	Greenhouse	38/28 to 33/27	Negative	−1 to 24%	Cao *et al.* (2009)
Seed-setting rate	–	28 and >35	Negative	−21%	Li *et al.* (2002)
Shoot biomass	Control chambers	30/25, 35/25, and 45/25	Negative	−98%	Yoshida *et al.* (1981)
Spikelet fertility	Field chambers	35	Negative	–	Morokuma and Yasuda (2004)
Spikelet fertility	Greenhouse	28 and 32 night temp.	Negative	− 72%	Mohammed and Tarpley (2009b)

Spikelet fertility	Phytotron	29, 35, 38 and 40	Negative	−80%	Satake and Yoshida (1978)
Spikelet formation	–	24–29	Negative	−40%	Yoshida and Parao (1976)
Spikelet, no. panicle^{-1}	–	22–31	Negative	−55%	Yoshida and Parao (1976)
Spikelet number/ panicle	Greenhouse	38/28 to 33/27	No effect	–	Cao *et al.* (2009)
Spikelet number/ panicle	TGC	30.4/21.2 to 39.7/22.1	Negative	−3%	Oh-e *et al.* (2007)
Spikelet number/ panicle	TGC	26–31	Negative	−12%	Kim *et al.* (1996b)
Spikelet numbers	Control chambers	26–35	Negative	−22 to −43%	Yoshida *et al.* (1981)
Spikelet numbers/m^2	TGC	30.4/21.2 to 39.7/22.1	Negative	−19 to −23%	Oh-e *et al.* (2007)
Spikelet numbers/m^2	TGC	26–31	Negative	−13 to −20%	Kim *et al.* (1996b)
Spikelet/floret sterility	–	35/30	Positive	600%	Sato *et al.* (1973)
Spikelet/floret sterility	–	23 and 30	Positive	23–70%	Nagato *et al.* (1966)
Spikelet/floret sterility	Glasshouse	24/19 to 39/34	Positive	1.4–48%	Tashiro and Wardlaw (1991b)
Spikelet/floret sterility	Phytotron	>38	Positive	85%	Yoshida (1981)
Spikelet/floret sterility	Glasshouse	36/31	Positive	+46%	Tashiro and Wardlaw (1991b)
Spikelet/floret sterility	TGC	Ambient +5	Positive	+51%	Prasad *et al.* (2006)
Spikelet/floret sterility	Sacxil growth cabinets	29.6 to 36.2	Positive	0.64–0.08	Jagadish *et al.* (2007)

(*Continued*)

Table 3 (*Continued*)

Parameter	Experimental facility	Temperature treatments (°C)	Association	Impact	Reference
Spikelet/floret sterility	–	–	Negative	–	Matsui (2009)
Spikelet/floret sterility	TGC	30.4/21.2 to 39.7/22.1	Positive	+235%	Oh-e *et al.* (2007)
Stem dry weight	Sunlit chambers	29/21 to 37/29	Negative	−30%	Manalo *et al.* (1994)
Vegetative biomass	TGC	Ambient +5	Negative	No change	Prasad *et al.* (2006)
Vegetative biomass	TGC	Ambient +4	Negative	−15%	Kobata and Uemuki (2004)
Total plant biomass (g)	Control chambers	30/25, 35/25, and 45/25	Negative	−98%	Yoshida *et al.* (1981)
White panicles	Control chambers	–	Positive	–	Yoshida *et al.* (1981)
White portion	–	–	Positive	–	Morita (2008)
Yield	TGC	26–31	Negative	−17 to −57%	Kim *et al.* (1996b)
Yield	Sunlit chambers	40/33 and 28/21	Negative	−100%	Baker *et al.* (1992)
Yield	Sunlit CEC	37/29 and 29/21	No effect	–	Ziska *et al.* (1996)
Yield	TGC	Ambient +4	Negative	−30%	Kobata and Uemuki (2004)
Yield	TGC	Ambient +5	Negative	−70%	Prasad *et al.* (2006)
Yield	Greenhouse	27 and 32	Negative	−85%	Mohammed and Tarpley (2009b)
Yield	Glasshouse	38/28 to 33/27	Negative	−3.9 to 27.5%	Cao *et al.* (2009)

GC, growth chamber; TGC, temperature gradient chamber; CEC, control environmental chamber; OTC, open-top chamber; OR, odds ratio.

temperature threshold for grain yield for some U.S. rice cultivars were between 32 and 35 °C (Baker *et al.*, 2005). According to Kim *et al.* (1996b), some yield will still be produced at daytime air temperature of 37 °C or as high as 39–40 °C.

As a result of high temperature, the extent of sterility can vary from a few empty glumes to the entire panicle having unfilled grains. Temperatures below 20 °C or above 35 °C and radiation lower than 200 cal cm^{-2} day^{-1} at anthesis can result in up to 40–60% sterility. Seed set and panicle weight of rice plants grown at higher temperatures (ambient +4 °C) are significantly reduced while green leaf area increased, relative to those plants grown at ambient temperatures (Lin *et al.*, 1997). The decline in the ratio of panicle weight to green leaf area suggests that the source/sink ratio may have been affected. The accumulation of leaf carbohydrate and increase in specific leaf weight indicate feedback inhibition. Decline in the number of filled grain per panicle decreases grain yield largely. High temperature induced infertility can make grain yield almost zero (Ziska *et al.*, 1996). The sink capacity under high temperature can be low due to the increase in the percentage of sterile spikelets and the reduced activity for starch synthesis can result in reduction in 1000-grain weight (Jeng *et al.*, 2003; Oh-e *et al.*, 2007).

Generally, the rice cultivars with high yield potential have grain weights in the range of 20–30 g and grain weight generally follows the order of maturity within a panicle, the first maturing grain being the heaviest. High temperature can increase the grain growth rate, but decrease the grain-filling period (Akita, 1989). The rice yield in the temperate or high altitude subtropical or tropical environments shows plasticity in the yield components and there are strong compensation mechanisms, particularly, for panicle and spikelet number in crops under tropical conditions. But the present cultivars for tropical environments do not have the capacity to produce sufficient assimilates to support the development of larger sink. Longer period of effective grain filling and longer duration of green leaf area are needed for active canopy photosynthesis to match the grain-filling duration. Hence, identification of yield components responsible for variations and sensitive to high temperature and improvement in those components becomes very pertinent to sustain or enhance grain yield in the future predicted warmer climate.

4.3. Grain quality

Grain quality is generally classified into four components: milling efficiency, grain shape and appearance, cooking and edibility characteristics, and nutritional quality. In most breeding programs, the major grain quality considerations are milling efficiency (head rice yield), shape and appearance (grain length before and after cooking, grain width and chalkiness), cooking and edibility characteristics (amylase content of the endosperm, gelatinization

temperature and aroma), and nutritional quality (protein, oil, and micronutrient content) (Resurreccion *et al.*, 1977). These quality characteristics are either subjective or objective and difficult to define as quality depends on consumer preferences and the intended end use of the product. Genetic grain quality determined by measurable physical and chemical characteristics includes gelatinization temperature, gel consistency, aroma, grain shape and size, bulk density, thermal conductivity, and equilibrium moisture content. Acquired traits include moisture content, color and chalkiness, purity, damage, cracked grains, immature grains, and milling-related characteristics (head rice recoveries, whiteness, and milling degree). In rice, the seed-to-seed cycle can be divided into different stages and high-temperature stress in each of these stages can result in changes in quality characteristics (Table 4).

Grain quality becomes poor when either high night or high day temperature is applied to the panicles or to the whole plants. Decreases in grain quality under high night temperature condition are not due to the deficit of carbohydrates in the leaves and the culms because exposure of the vegetative parts to this temperature condition does not decrease grain quality (Morita *et al.*, 2004). When nighttime temperature increases from 18 to 30 °C from 12 to 5 a.m., head rice yields significantly decrease, grain dimensions generally decrease, and the amylase content gets lowered, but the grain mass, total brown rice lipid, and protein contents do not vary, albeit with some differences among rice cultivars (Cooper *et al.*, 2008). High night temperatures can reduce grain widths. Elevated [CO_2] can significantly increase brown rice yield, but high night temperature decreases yield, with a significant interaction of [CO_2] and night temperature (Cheng *et al.*, 2009).

High head rice yield is an important criterion for measuring milled rice quality and depends on varietal characteristics, crop management practices, and drying and milling process. High temperature causes interruption during the final stages of grain filling, resulting in excessive chalkiness. Likewise, high-temperature stress during ripening results in starch with a higher gelatinization temperature. The quality characteristics of milled rice are classified both physically and chemically. Across the RH range of 25–85%, high air temperature produces higher amounts of broken grains. At higher moisture content levels, milled rice sustains more extensive stress crack damage at low RH conditions and less stress crack damage at high RH conditions, relative to milled rice at lower moisture content levels (Siebenmorgen *et al.*, 1998).

4.4. Seed longevity and cooking characteristics

4.4.1. Seed longevity

Rice produces orthodox seeds, which can be dried and stored at low temperatures to prolong viability. Longevity, defined as the period during which seeds retain viability after harvesting, is generally evaluated by the

Table 4 Effect of high-temperature stress on grain quality parameters

Grain quality parameter	Experimental facility	Temperature treatments (°C)	Impact	Association	Reference
Abortive kernels	Glasshouse	24/19 to 39/34	Positive	18.40%	Tashiro and Wardlaw (1991b)
Air spaces between grain	Glasshouse	24/19 to 39/34	Positive	–	Tashiro and Wardlaw (1991b)
Brown rice	TGC	30.4/21.2 to 39.7/22.1	Negative	−32%	Oh-e *et al.* (2007)
Chalky kernels	Field	28–26/21–26 and 25/32/19–24	Positive	30–50%	Nagato *et al.* (1961)
Chalky kernels	Glasshouse	24/19 to 39/34	Positive	–	Tashiro and Wardlaw (1991b)
Chalky kernels	Control chambers	33/28 or 25/20	Positive	–	Yamakawa *et al.* (2007)
Chalky kernels	Glasshouse	22/34, 34/22, and 22/22	Positive	–	Morita *et al.* (2005)
Chalky kernels	Phytotron	35/18 to 35/30 night temperature	Positive	0.6–34%	Cooper *et al.* (2008)
Cracking grain	–	–	Positive	–	Morita (2008)
Deep ditch in kernel	–	–	Positive	–	Morita (2008)
Dorsoventral ratio of the grain	Field	23 and 30	Negative	−5%	Nagato *et al.* (1966)
Endosperm area of cross section	Glasshouse	22/34, 34/22, and 22/22	Negative	−9%	Morita *et al.* (2005)
Endosperm cell area	Glasshouse	22/34, 34/22, and 22/22	Negative	−30%	Morita *et al.* (2005)

(*Continued*)

Table 4 (*Continued*)

Grain quality parameter	Experimental facility	Temperature treatments (°C)	Impact	Association	Reference
Endosperm cell number	Glasshouse	22/34, 34/22, and 22/22	Positive	20%	Morita *et al.* (2005)
Grain fissuring	Field and Chamber	30/25	Positive	5–55%	Nagata *et al.* (2004)
Grain length	Glasshouse	24/19 to 39/34	Negative	−2%	Tashiro and Wardlaw (1991b)
Grain length	Growth chamber	35/24 and 35/18	No effect	–	Counce *et al.* (2005)
Grain length	Glasshouse	22/34, 34/22, and 22/22	No effect	–	Morita *et al.* (2005)
Grain length	Phytotron	35/18 to 35/30	Negative	−2 to −4%	Cooper *et al.* (2008)
Grain thickness	Glasshouse	24/19–39/34	Negative	−17%	Tashiro and Wardlaw (1991b)
Grain thickness	Growth chamber	35/24 and 35/18	No effect	–	Counce *et al.* (2005)
Grain thickness	Glasshouse	22/34, 34/22, and 22/22	No effect	–	Morita *et al.* (2005)
Grain thickness	Phytotron	35/18 to 35/30 night temperature	Negative	−0.5 to −1%	Cooper *et al.* (2008)
Grain width	Glasshouse	24/19 to 39/34	Negative	−10%	Tashiro and Wardlaw (1991b)
Grain width	Growth chamber	35/24 and 35/18	No effect	–	Counce *et al.* (2005)
Grain width	Glasshouse	22/34, 34/22, and 22/22	No effect	–	Morita *et al.* (2005)
Grain width	Phytotron	35/18 to 35/30 night temperature	Negative	−2 to −10%	Cooper *et al.* (2008)
Head rice yield	Growth chamber	35/24 and 35/18	Negative	−10%	Counce *et al.* (2005)
Head rice yield	Phytotron	35/18 to 35/30 night temperature	Negative	−7 to −23%	Cooper *et al.* (2008)
Kernel breadth	Field	28–26/21–26 and 25/32/19–24	Negative	−1 to −2%	Nagato *et al.* (1961)

Kernel breaking force	Phytotron	35/18 to 35/30 night temperature	Negative	−37 to −27%	Cooper *et al.* (2008)
Kernel length	Field	28–26/21–26 and 25/32/19–24	Negative	−1 to −5%	Nagato *et al.* (1961)
Kernel mass	Phytotron	35/18 to 35/30 night temperature	No effect	–	Cooper *et al.* (2008)
Kernel weight	Field	28–26/21–26 and 25/32/19–24	Negative	3–6%	Nagato *et al.* (1961)
Milky white rice kernels	Field	28–26/21–26 and 25/32/19–24	Positive	30–50%	Nagato *et al.* (1961)
Milky white rice kernels	Field	23 and 30	Positive	8–85%	Nagato *et al.* (1966)
Milky white rice kernels	Glasshouse	24/19 to 39/34	Positive	2.4–86.3%	Tashiro and Wardlaw (1991b)
Milky white rice kernels	Plastic film	23 and 29	Positive	1–16%	Kobata *et al.* (2004)
Milled rice	Growth chamber	35/24 and 35/18	No effect	–	Counce *et al.* (2005)
Opaque kernels	Glasshouse	24/19 to 39/34	Positive	73.70%	Tashiro and Wardlaw (1991b)
Palatability	–	–	Negative	–	Morita (2008)
Palatability	Control chambers	33/28 or 25/20	Negative	−1 to −4.8 times	Yamakawa *et al.* (2007)
Parthenocarpic kernels	Glasshouse	36/3l	Positive	15.80%	Tashiro and Wardlaw (1991b)
Perfect kernel ratio	Control chambers	33/28 or 25/20	Negative	−61 to −74%	Yamakawa *et al.* (2007)
Quality of rice grain	–	–	Negative	–	Morita *et al.* (2004)
Quality of rice grain	Field poly-house	35	Negative	–	Ishizaki (2006)
Starch granules	Greenhouse	31/26 and 40/32	Positive	–	Zakaria *et al.* (2002)

(*Continued*)

Table 4 (*Continued*)

Grain quality parameter	Experimental facility	Temperature treatments (°C)	Impact	Association	Reference
Stickiness	Control chambers	33/28 or 25/20	Positive	1–4 times	Yamakawa *et al.* (2007)
Thickness of bran layer in kernel	Field	28–26/21–26 and 25/32/19–24	Positive	5–6%	Nagato *et al.* (1961)
Thickness of bran layer in kernel	–	23 and 30	Positive	1–15%	Nagato *et al.* (1966)
Thickness of aleurone cell layer	Field	28–26/21–26 and 25/32/19–24	Positive	2–5%	Nagato *et al.* (1961)
Thickness of aleurone cell layer	Field	23 and 30	Positive	2–28%	Nagato *et al.* (1966)
Total solid content	Field	23 and 30	Negative	−20 to −40%	Nagato *et al.* (1966)
Water uptake ratio	Field	23 and 30	Negative	−7 to −20%	Nagato *et al.* (1966)
White core kernel	Glasshouse	24/19 to 39/34	Positive	7.30%	Tashirao and Wardlaw (1991b)
White-back kernel	Field	>27	Positive	–	Wakamatsu *et al.* (2007, 2008)
White-back kernel	Glasshouse	24/19 to 39/34	Positive	11.9–34.8%	Tashiro and Wardlaw (1991b)
White-based kernel	Field	23 and 30	Positive	2.4–13.6%	Nagato *et al.* (1966)
Yield after polishing	Control chambers	33/28 or 25/20	Negative	−2.6 to −6.1%	Yamakawa *et al.* (2007)

GC, growth chamber; TGC, temperature gradient chamber; CEC, control environmental chamber; OTC, open-top chamber.

germination ratio, which decreases with the loss of seed viability during storage and the seed longevity period includes the seed dormancy period. Both seed longevity and dormancy are affected by pre- and postharvest environmental conditions (Ellis *et al.*, 1993; Kameswara Rao and Jackson, 1996). Temperature and RH (or seed moisture content) are two important factors that affect longevity during storage (Roberts, 1972). Much is known about preharvest factors (seed production environment and degree of seed maturity) that influence longevity. Generally, cool sites with low RH are known to be conducive to the production of good quality seeds (Andrews, 1982). Seeds attain maximum viability and vigor at physiological maturity, a stage when seeds reach maximum dry weight, and aging declines viability and vigor (Harrington, 1972). Immature seeds lose viability faster than mature seeds under similar storage conditions. The maximum potential longevity in developing seeds is attained some time after the end of the grain-filling period, defined as mass maturity (Ellis and Pieta Filho, 1992). In rice, improvement in longevity subsequent to mass maturity is influenced by the seed production environment and genotype (Ellis and Hong, 1994; Ellis *et al.*, 1993). The potential longevity of the *japonica* cultivars is significantly less when produced under a warm seed production regime (32/24 °C) than in a cooler regime (28/20 °C). The maximum potential longevity of the seeds of *japonica* cultivars is less than that of the *indica* cultivars. Alterations in rice quality characteristics begin under field conditions and continue after harvest. In addition, there are changes in rice quality as a result of aging, which are due to enzymatic reactions involving protein, starch, and lipid. During postharvest storage, moisture content, temperature, and time are most influential on the chemical, physical, and functional qualities of rice, and the rate and nature of these changes are primarily temperature dependent.

4.4.2. Cooking characteristics

Typically, rice grains are consumed as cooked rice food, with only a small amount being used to make ingredients for processed foods. The composition of rice grains is 90% starch and approximately 2% lipids, 6–8% proteins, and 1% minerals. The proportions and structures of two types of starch (amylose content and the fine structure of amylopectin) are key determinants that affect cooking quality of rice. The parameters such as apparent amylase content, gel consistency, gelatinization temperature, and the rapid visco analyzer (RVA) profile are commonly used to define eating and cooking qualities of rice. Storage results in numerous changes in chemical and physical properties of rice (Jang *et al.*, 2009). Meullenet *et al.* (2000) reported that storage temperature and duration affected all flavor and texture attributes of rice stored as paddy (rice grains in their natural and unprocessed state). Following storage at high temperatures, the textural profile of the cooked rice grain changes with increased hardness, reduced

adhesiveness, lower leaching of starch components, particularly amylase, and rougher surfaces. Patindol *et al.* (2005) reported that starch structure and physicochemical properties were affected by rough rice stored at three temperatures (4, 21, and 38 °C) for 9 months. High temperature even after flowering decreases final viscosity and the amylose content to some extent. On the contrary, high temperatures can increase the maximum viscosity and breakdown values and hardness versus adhesion ratio of cooked rice (Tanaka *et al.*, 2009).

Rice grains have a gelatinization temperature of 65–80 °C, at which rice starch begins to gelatinize and take up water. The gelatinization process which can be divided into two steps, swelling of the amorphous region and disruption of the crystalline region, is influenced by high-temperature storage: the breaking point temperature is increased suggesting that energy for the disorder of these two regions of starch in rice stored at high temperature (Zhou *et al.*, 2010). The effects of storage on thermal properties are often associated with the interactions between starch and nonstarch components following storage. More likely, the changes in cell wall remnants and proteins are responsible for the changes in rice thermal properties during storage. All the quality parameters of rice can be affected by the growth conditions of plants, in particular, high temperatures during grain filling, field fertilization, and moisture content during harvest.

5. Mechanisms of High-Temperature Injury

Environmental factors are not always at optimal conditions and may reach a level which represents stress for plants. Stress can cause variable effects at all functional levels of plants. When plants are exposed to stresses, there are decreases in activities and energy for growth and development. Crop losses can occur eventually due to stresses. High-temperature stress affects various biochemical and physiological processes, which are listed in Tables 5 and 6. High-temperature stress will have negative impact on the growth and development of plants, especially during reproduction. The stress due to high temperature can severely limit plant productivity, causing extensive economic loss. Understanding adaptive mechanisms in plants is critical for identifying and developing high-temperature-tolerant cultivars (Tables 5 and 6).

5.1. Photosynthesis

Photosynthesis is sensitive to high-temperature stress, and maintenance of high photosynthetic capacity is critical for tolerance. The temperature optimum for photosynthesis in rice is broad, presumably because rice plants

Table 5 Effect on important physiological processes and/or their association with high temperature in rice

Physiological process	Experimental facility	Temperature treatment (°C)	Impact	Association	Reference
Chlorophyll fluorescence (F_v/F_m)	Growth chambers	26, 35, 40, and 45	Negative	−25%	Han *et al.* (2009)
Dark respiration rate (net)	Greenhouse	27 and 32 night temperature	Positive	27%	Mohammed and Tarpley (2009b)
Dark respiration rate of leaf	Greenhouse	27 and 32 night temperature	Positive	7%	Mohammed and Tarpley (2009b)
Dark respiration rate of leaf	TGC	30.4/21.2 to 39.7/22.1	Positive	20%	Oh-e *et al.* (2007)
Dark respiration rate of panicle	Greenhouse	27 and 32 night temperature	Positive	30%	Mohammed and Tarpley (2009b)
Dark respiration rate of panicle	TGC	30.4/21.2 to 39.7/22.1	Negative	−41%	Oh-e *et al.* (2007)
Dark respiration rate of stem	Greenhouse	27 and 32 night temperature	Positive	25%	Mohammed and Tarpley (2009b)
Dark respiration rate of stem	TGC	30.4/21.2 to 39.7/22.1	Positive	30%	Oh-e *et al.* (2007)
Dark respiration rate of whole plant	Greenhouse	27 and 32 night temperature	Positive	22%	Mohammed and Tarpley (2009b)
Dark respiration rate of whole plant	TGC	30.4/21.2 to 39.7/22.1	Positive	11%	Oh-e *et al.* (2007)
Evapotranspiration	Sunlit chambers	28/21, 34/27, and 40/33	Positive	25–33%	Baker and Allen (1993b)

(*Continued*)

Table 5 (*Continued*)

Physiological process	Experimental facility	Temperature treatment (°C)	Impact	Association	Reference
Leaf area index (LAI)	TGC	26–31	Positive	30–50%	Kim *et al.* (1996b)
Leaf temperature	Greenhouse	40/21 and 30/21	Positive	16–40%	Cao *et al.* (2009)
LAR	Growth chambers	19/16, 25/19, 30/24, and 37/31	Negative	−16%	Nagai and Makino (2009)
LWR	Growth chambers	19/16, 25/19, 30/24, and 37/31	Positive	25%	Nagai and Makino (2009)
NAR	Growth chambers	19/16, 25/19, 30/24, and 37/31	Negative	−8 to −15%	Nagai and Makino (2009)
Net canopy photosynthesis	Sunlit CEC	28/21, 34/27, and 40/33	Negative	−38%	Rowland-Bamford *et al.* (1996)
NUE for GR (mmol N^{-1} day^{-1})	Growth chambers	19/16, 25/19, 30/24, and 37/31	Negative	−45%	Nagai and Makino (2009)
Photosynthetic assimilation rate	Growth chambers	19/16, 25/19, 30/24, and 37/31	No effect	–	Nagai and Makino (2009)
Photosynthetic rate	OTC	25.6 and 29.5	Negative	−14%	Lin *et al.* (1997)
Photosynthetic rate	Sunlit chambers	28/21, 34/27, and 40/33	Positive	25–33%	Baker and Allen (1993b)
Photosynthetic rate	TGC	Ambient +5	Negative	−14%	Prasad *et al.* (2006)

Photosynthetic rate	SPAR	28/18, 34/24, and 40/30	Negative	−15 to 25%	Gesch *et al.* (2003)
Photosynthetic rate	TGC	30.4/21.2 to 39.7/22.1	Negative	−40%	Oh-e *et al.* (2007)
Photosynthetic rate	Growth chambers	19/16, 25/19, 30/24, and 37/31	Negative	−20 to −40%	Nagai and Makino (2009)
Photosynthetic rate	Greenhouse	40/21 and 30/21	Negative	2.6–16%	Cao *et al.* (2009)
Photosynthetic rate	Greenhouse	27 and 32 night temperature	Negative	−2.2%	Mohammed and Tarpley (2009b)
Photosynthetic rate	SPAR	32, 35, and 38	Negative	−15%	Vu *et al.* (1997)
Photosystem I and (PSII) activity	Incubator	40 and 25	Negative	−45%	Vani *et al.* (2001)
Relative membrane injury	TGC	Ambient +5	No effect	–	Prasad *et al.* (2006)
Relative membrane injury	CEC	30/25 and 37/30	Positive	15–42%	Zhang *et al.* (2006, 2009)
Relative membrane injury	Greenhouse	27 and 32	Positive	45%	Mohammed and Tarpley (2009b)
Relative membrane injury	CEC	28 and 42	Positive	15–42%	Lee *et al.* (2007)
Respiration	–	22/34, 34/22, and 22/22	Positive	–	Morita *et al.* (2004)
Respiration rate of kernels	Outdoor and chamber	Ambient and 35	Negative	−15 to −20%	Inaba and Sato (1976a)
Respiration rate of rough rice	Oven	20–80	Positive	84%	Dillahunty *et al.* (2000)

(*Continued*)

Table 5 (*Continued*)

Physiological process	Experimental facility	Temperature treatment (°C)	Impact	Association	Reference
RGR	Growth chambers	19/16, 25/19, 30/24, and 37/31	Negative	−12 to −38%	Nagai and Makino (2009)
RGR	Control chambers	30/25, 35/25, and 45/25	Negative	−33%	Yoshida *et al.* (1981)
SLA (m^2 g^{-1})	Growth chambers	19/16, 25/19, 30/24, and 37/31	Negative	−46%	Nagai and Makino (2009)
Starch accumulation in kernel	Control chambers	33/28 or 25/20	Positive	10%	Yamakawa *et al.* (2007)
Stomatal conductance	Greenhouse	28 and 32 night temperature	Negative	−5%	Mohammed and Tarpley (2009b)
Water loss	Sunlit chambers	28/21, 34/27, and 40/33	Positive	21–40%	Baker and Allen (1993b)
Water-use efficiency	Sunlit chambers	28/21, 34/27, and 40/33	Negative	−15 to 75%	Baker and Allen (1993b)
Yellowing of spikelet	Outdoor and chamber	Ambient and 35	Positive	–	Sato and Inaba (1976a)

LAR, leaf area ratio; LWR, leaf weight ratio; NAR, net assimilation rate; NUE, nitrogen-use efficiency; RGR, relative growth rate; SLA, specific leaf area; GC, growth chamber; TGC, temperature gradient chamber; CEC, control environmental chamber; OTC, open-top chamber; SPAR, Soil–Plant–Atmosphere-Research units.

Table 6 Changes in biochemical parameters in rice under high-temperature condition

Biochemical parameter	Experimental facility	Temperature treatments (°C)	Impact	Association	Reference
ABA in anther	Phytotron	32 and 39	Positive	35%	Tang *et al.* (2008)
Amylopectin chain length	Growth chamber	35/24 and 35/18	No effect	–	Counce *et al.* (2005)
Amylopectin chain length	Phytotron	35/18 to 35/30	Positive	–	Cooper *et al.* (2008)
Amylopectin chain length—long	Control chambers	33/28 or 25/20	Positive	10%	Yamakawa *et al.* (2007)
Amylopectin chain length—short	Control chambers	33/28 or 25/20	Negative	−40%	Yamakawa *et al.* (2007)
Ascorbate peroxidase (APX)	Growth chambers	26, 35, 40, and 45 for 48 h	Positive	211%	Han *et al.* (2009)
ATPase in grain	Greenhouse	40/21 and 30/21	Negative	−7.5 to −8.75%	Cao *et al.* (2009)
Carbohydrate in panicle	Field and chamber	25/20 and 35/30	Negative	−10–15%	Sato and Inaba (1973)
Carbohydrate in straw	Field and chamber	25/20 and 35/30	Positive	4–6%	Sato and Inaba (1973)
Carbohydrate in straw	Field	28–26/21–26 and 25/32/19–24	Positive	25–50%	Nagato *et al.* (1966)
Carbohydrate in straw	–	35/30	Positive	2.5 times	Sato *et al.* (1973)
Carbohydrate in straw	Field	28–26/21–26 and 25/32/19–24	Positive	25–30%	Nagato *et al.* (1966)
CAT (catalase)	Greenhouse	40/21 and 30/21	Positive	11.6–41.3%	Cao *et al.* (2009)
Chlorophyll (*a*)	Greenhouse	28 and 32 night temperature	Negative	−12%	Mohammed and Tarpley (2009b)

(*Continued*)

Table 6 (*Continued*)

Biochemical parameter	Experimental facility	Temperature treatments (°C)	Impact	Association	Reference
Chlorophyll (*a*/*b*)	Growth chambers	19/16, 25/19, 30/24, and 37/31	Negative	−10%	Nagai and Makino (2009)
Chlorophyll (*b*)	Greenhouse	28 and 32 night temperature	No effect	–	Mohammed and Tarpley (2009b)
Ear carbon content (g plant^{-1})	Glasshouse	28/23, 35/33, and 38/26	No effect	–	Ito *et al.* (2009)
Ear carbon content (mg g^{-1} DW)	Glasshouse	28/23, 35/33, and 38/26	No effect	–	Ito *et al.* (2009)
Ear nitrogen content (g plant^{-1})	Glasshouse	28/23, 35/33, and 38/26	Negative	−17%	Ito *et al.* (2009)
Ear nitrogen content (mg g^{-1} DW)	Glasshouse	28/23, 35/33, and 38/26	Negative	–14%	Ito *et al.* (2009)
Ear sugar concentration (mg g^{-1} DW)	Glasshouse	28/23, 35/33, and 38/26	Positive	100%	Ito *et al.* (2009)
Free proline in anther	Phytotron	32 and 39	Negative	−30%	Tang *et al.* (2008)
GAs in anther	Phytotron	32 and 39	Negative	−68%	Tang *et al.* (2008)
Heat shock proteins	Growth chamber	43–49	Positive	–	Fourré and Lhoest (1989)
Heat shock proteins	Growth chamber	27 and 42	Positive	–	Murakami *et al.* (2004)
Heat shock proteins	Growth chamber	28 and 42	Positive	–	Hu *et al.* (2009)
IAA in anther	Phytotron	32 and 39	Negative	−55%	Tang *et al.* (2008)
Kernel carbon concentration (%)	Glasshouse	24/19 to 39/34	Positive	1%	Tashiro and Wardlaw (1991a)

Kernel carbon content (mg kernel^{-1})	Glasshouse	24/19 to 39/34	Negative	−39%	Tashiro and Wardlaw (1991a)
Kernel nitrogen concentration (%)	Glasshouse	24/19 to 39/34	Positive	10%	Tashiro and Wardlaw (1991a)
Kernel nitrogen content (mg kernel^{-1})	Glasshouse	24/19 to 39/34	Negative	−30%	Tashiro and Wardlaw (1991a)
Leaf total nonstructural carbohydrate	Sunlit CEC	28/21, 34/27, and 40/33	Negative	−25%	Rowland-Bamford *et al.* (1996)
Leaf sucrose concentration	Sunlit CEC	28/21, 34/27, and 40/33	Negative	−7%	Rowland-Bamford *et al.* (1996)
Lipid content inkernel	Phytotron	35/18 to 35/30	No effect	–	Cooper *et al.* (2008)
Malondialdehyde content	CEC	30/25 and 37/30	Positive	16–38%	Zhang *et al.* (2009)
MDA (malondialdehyde)	Greenhouse	40/21 and 30/21	Positive	14.7–56.5%	Cao *et al.* (2009)
Nitrogen in panicle	–	35/30	Positive	10–50%	Sato *et al.* (1973)
Panicle total NSC	Sunlit CEC	28/21, 34/27, and 40/33	Negative	−85%	Rowland-Bamford *et al.* (1996)
POD (peroxidase)	Greenhouse	40/21 and 30/21	Positive	59.9–97.4%	Cao *et al.* (2009)
Protein content in kernel	Phytotron	35/18 to 35/30	No effect	–	Cooper *et al.* (2008)
Protein content in leaves	SPAR	28/21, 34/27, and 40/33	Negative	−19 to 48%	Gesch *et al.* (2003)

(*Continued*)

Table 6 (*Continued*)

Biochemical parameter	Experimental facility	Temperature treatments (°C)	Impact	Association	Reference
Root carbon content (g plant^{-1})	Glasshouse	28/23, 35/33, and 38/26	Positive	36%	Ito *et al.* (2009)
Root carbon content (mg g^{-1} DW)	Glasshouse	28/23, 35/33, and 38/26	No effect	–	Ito *et al.* (2009)
Root nitrogen content (g plant^{-1})	Glasshouse	28/23, 35/33, and 38/26	Positive	43%	Ito *et al.* (2009)
Root Nitrogen content (mg g^{-1} DW)	Glasshouse	28/23, 35/33, and 38/26	No effect	–	Ito *et al.* (2009)
Root activity	Greenhouse	40/21 and 30/21	Negative	−12 to −26%	Cao *et al.* (2009)
Root sugar concentration (mg g^{-1} DW)	Glass house	28/23, 35/33, and 38/26	Positive	28%	Ito *et al.* (2009)
Rubisco (g m^{-2})	Growth chambers	19/16, 25/19, 30/24, and 37/31	Negative	−25 to −45%	Nagai and Makino (2009)
Rubisco activity in leaves	Growth chambers	35, 40, 45, and 50	Negative	−60 to 80%	Bose *et al.* (1999)
Rubisco activity in leaves	SPAR	32, 35, and 38	Negative	−13%	Vu *et al.* (1997)
Rubisco activity in leaves	SPAR	28/18, 34/24, and 40/30	Negative	−25 to 45%	Gesch *et al.* (2003)
Shoot carbon content (g plant^{-1})	Glasshouse	28/23, 35/33, and 38/26	Positive	11%	Ito *et al.* (2009)

Shoot carbon content (mg g^{-1} DW)	Glasshouse	28/23, 35/33, and 38/26	No effect	–	Ito *et al.* (2009)
Shoot nitrogen content (mg g^{-1} DW)	Glasshouse	28/23, 35/33, and 38/26	Negative	−20%	Ito *et al.* (2009)
Shoot sugar concentration (mg g^{-1} DW)	Glasshouse	28/23, 35/33, and 38/26	Positive	375%	Ito *et al.* (2009)
Shoot nitrogen content (g $plant^{-1}$)	Glasshouse	28/23, 35/33, and 38/26	Negative	−9%	Ito *et al.* (2009)
SOD (superoxide dismutase)	Greenhouse	40/21 and 30/21	Positive	51.8–93.4%	Cao *et al.* (2009)
Soluble proteins in anther	Phytotron	32 and 39	Negative	−39%	Tang *et al.* (2008)
Stem/culm total NSC	Sunlit CEC	28/21, 34/27, and 40/33	Negative	−40%	Rowland-Bamford *et al.* (1996)
SDS activity at rachilla	Growth chamber	30–35	Negative	–	Inaba and Sato (1976)
Sucrose accumulation rate in leaf	Sunlit CEC	28/21, 34/27, and 40/33	Negative	−40%	Rowland-Bamford *et al.* (1996)
Sucrose concentration in stem	Glasshouse	28/23, 35/33, and 38/26	Positive	30–40%	Ito *et al.* (2009)
TBARS	CEC	28 and 42	Positive	12%	Lee *et al.* (2007)
Total antioxidant activity	Greenhouse	28 and 32 night temperature	No effect	–	Mohammed and Tarpley (2009b)
Total carbon content (g $plant^{-1}$)	Glasshouse	28/23, 35/33, and 38/26	Positive	28%	Ito *et al.* (2009)

(*Continued*)

Table 6 (*Continued*)

Biochemical parameter	Experimental facility	Temperature treatments (°C)	Impact	Association	Reference
Total chlorophyll content	Greenhouse	28 and 32 night temperature	Negative	−10%	Mohammed and Tarpley (2009b)
Total chlorophyll content	Growth chambers	19/16, 25/19, 30/24, and 37/31	Negative	−6 to −13%	Nagai and Makino (2009)
Total leaf nitrogen (mmol N m^{-2})	Growth chambers	19/16, 25/19, 30/24, and 37/31	Negative	−20 to −40%	Nagai and Makino (2009)
Total nitrogen content (g $plant^{-1}$)	Glasshouse	28/23, 35/33, and 38/26	No effect	–	Ito *et al.* (2009)
Total nitrogen content (mmol N $plant^{-1}$)	Growth chambers	19/16, 25/19, 30/24, and 37/31	Negative	−10%	Nagai and Makino (2009)
Total sugar concentrations in kernel	Field and chamber	25/20 and 35/30	Positive	50–60%	Sato *et al.* (1973)
Total sugar concentrations in straw	Field and chamber	25/20 and 35/30	Positive	10%	Sato *et al.* (1973)

TBARS, thiobarbituric acid reactive substance; SDS, sucrose dehydrogenase; NSC, nonstructural carbohydrate; IAA, indole-3-acetic acid; Gas, gibberellic acids; ABA, abscisic acid; GC, growth chamber; TGC, temperature gradient chamber; CEC, control environmental chamber; OTC, open-top chamber; SPAR, Soil–Plant–Atmosphere-Research units.

have adapted to a relatively wide range of thermal environments. In rice, there is little temperature effect on leaf photosynthesis from 20 to 40 °C (Egeh *et al*., 1992). A 1–2 °C increase in average temperature is not likely to have a substantial impact on leaf photosynthetic rates. However, variability in leaf photosynthetic rates within rice cultivars and high photosynthetic rates at high temperatures do not necessarily support high rates of dry matter accumulation. Although global warming is not likely to affect photosynthetic rates per unit leaf area of a closed canopy over the next century, very high temperatures can inhibit photosynthesis. The inhibition of photosynthesis due to heat stress can be associated with interruption of photosynthetic electron transport, reduction in photochemical efficiency in PSII, and CO_2 fixation and partitioning.

Alterations in various photosynthetic attributes are good indicators of plant's thermotolerance as they show correlations with growth. Wise *et al*. (2004) suggested that the photochemical reactions in thylakoid lamellae and carbon metabolism in the stroma of chloroplast are the primary sites of injury at high temperatures in cotton. In a field study at the experimental farm of the IRRI, Philippines, Egeh *et al*. (1992) subjected four rice genotypes (N22, IR52, IR20, and IR46) to high temperature using open-top plastic chambers at 30 days after transplanting and investigated the temperature response of gas exchange traits. Simultaneously, the same genotypes were subjected to four day/night temperature regimes of 29/21, 33/25, 37/29, and 41/33 °C in a phytotron. They found that increased temperature reduced leaf conductance and transpiration rate of N22 and IR52, but increased leaf and canopy temperature of both genotypes in the open-top plastic chambers. Transpiration rate, leaf conductance, and intercellular [CO_2] were greater for N22 than for the other genotypes at 41/33 °C and contributed to the high-temperature tolerance of N22. The leaf photosynthesis of rice increased from the lowest (22 °C) to the intermediate temperature (32 °C) and then decreased in plants grown at 42 °C. The activities of the organelles (protoplast and chloroplast) were found to decrease slowly but steadily from lowest (22 °C) to the highest temperature (42 °C). The related responses of whole plants, protoplasts, chloroplasts, and thylakoids to high temperature provide a strong evidence of the involvement of a common component of photochemistry. Al-Khatib and Paulsen (1999) observed that temperature had no effect on stomatal conductance and internal [CO_2] in rice, suggesting the noninvolvement of stomatal effects in the changes in photosynthetic rates with temperature. High temperatures reduce chlorophyll fluroscence (F_v) in attached leaves, protoplasts, chloroplasts, or thylakoids of rice (Al-Khatib and Paulsen, 1999). Injury to PSII in photosynthetic organelles and thylakoids and the match between these profiles and F_v, an indicator of damage to PSII and the kinetics of injury over time suggest that the photosystem is susceptible to high-temperature damage in rice.

Single leaves of rice show a cooperative enhancement of photosynthetic rate with elevated [CO_2] and temperature during tillering, relative to the elevated [CO_2] (Lin *et al.*, 1997). At flowering stage, photosynthetic stimulation by elevated [CO_2] appeared to be accompanied by a reduction in ribulose-1,5-biphosphate carboxylase/oxygenase (Rubisco [EC 4.1.1.39]) activity and/or concentration as evidenced by the reduction in the assimilation at a standard internal [CO_2] (Ci). High temperature can reduce photosynthetic rate by 40–60% at mid-ripening, leading to more rapid senescence of the flag leaf (Oh-e *et al.*, 2007). When characterizing the temperature responses of photosynthesis and growth in rice grown hydroponically under day/night temperature regimes of 13/10, 19/16, 25/19, 30/24, and 37/31 °C, the optimal temperature was found to be at 30–35 °C (Nagai and Makino, 2009). The leaf photosynthesis rates were found to be highest under midday temperatures of 35 °C, but declined with higher or lower midday growth temperatures, under both low (350 μmol mol^{-1}) and high (660 μmol mol^{-1}) levels of [CO_2] (Vu *et al.*, 1997). There also exists intraspecies variation in rice responses to increasing temperature under elevated [CO_2] (Gesch *et al.*, 2001). High temperatures also can lead to greater sink demand due to increased growth and respiration, resulting in a more rapid use of assimilates. This too is expected to enhance the stimulation of photosynthesis by elevated [CO_2] at high temperatures.

Photosynthetic processes of rice are negatively affected by high temperatures, but to a lesser extent than reproductive development. In an experiment with rice (cv. IR72) grown for a full season in sunlit, controlled-environment chambers (CECs) at 350 (ambient) and 700 (double-ambient, elevated) μmol CO_2 mol^{-1}, and under daytime maximum/nighttime minimum air temperature regimes ranging from 28/18 to 48/38 °C and soil water deficit, Vu *et al.* (2007) tested whether elevated [CO_2], high temperature, or severe drought stress would induce changes in the kinetic behavior [$K_m(CO_2)$] of rubisco. They found that the leaf CO_2 exchange rate (CER) of rice was increased by CO_2 enrichment, but was decreased by high temperature and drought; the [CO_2]-enriched plants not only outperformed ambient-[CO_2]-grown plants at the optimum growth temperature (32/22 °C) for photosynthesis but also compensated much better for the adverse effects of high temperatures on CER. High temperature, elevated [CO_2], and drought have been found to reduce the initial (nonactivated) and total (HCO_3-/Mg^{2+} activated) activities as well as the activation state of midday-sampled leaf rubisco. The responsiveness of the carbon balance of C_3 plants such as rice to increased [CO_2] will increase as temperature increases, primarily due to the interactive effects that elevated [CO_2] and temperature have on rubisco kinetics (Gesch *et al.*, 2003). Photorespiration will increase with temperature, largely because of the reduction in the specificity of rubisco for CO_2 and its activation. Since increasing [CO_2] will partially depress photorespiration, theoretically,

the enhancement of net photosynthesis is expected to increase with temperature at atmospheric [CO_2] predicted for the future. The ratio of chlorophyll *a*/*b* decreases with increasing temperature, and chlorophyll *b* content is greater in rice plants grown at high temperature (Nagai and Makino, 2009). Although high temperatures can stimulate plant growth to some extent, they also speed up development thus shortening the life cycle. Under high temperatures, tissues and organs have less time to acquire photoassimilates, which can result in fewer and/or smaller organs leading to less biomass accumulation.

The light-saturated photosynthetic rates of leaves are highly correlated with atmospheric [CO_2], and temperature dependence of photosynthesis varies with the growing temperature, even within a genotype (Oh-e *et al.*, 2007). With changes in growth temperature, rice may show considerable phenotypic plasticity in its photosynthetic characteristics. Temperature dependence of photosynthesis is sensitive to the [CO_2] and the optimal temperature increases with [CO_2]. Lin *et al.* (1997) showed a cooperative enhancement of photosynthetic rate with temperature under elevated [CO_2] during tillering stage relative to the elevated [CO_2] condition alone. However, after flowering, the degree of photosynthetic stimulation by elevated [CO_2] was reduced under high temperature (ambient + 4 °C). This increasing insensitivity to [CO_2] under high temperature was attributed to the reduction in rubisco activity. The acclimation of photosynthesis to increasing temperatures may occur at the whole-leaf level or in isolated chloroplasts. The physiological acclimation may result in increases in both the heat tolerance and the temperature optimum for net CO_2 uptake of leaves.

Baker *et al.* (2005) observed that at 700 μmol mol^{-1} CO_2, the temperature optimum for canopy net assimilation (A_{can}) appeared to be near 28–32 °C, with higher- or lower-temperature treatments resulting in lower A_{can}. High nighttime temperature (42 ± 1 °C) decreases the net photosynthetic rate (P_n), the apparent quantum yield (AQY), the photochemical efficiency of PSII (F_v/F_m), the quantum yield of PSII electron transport (Φ_{PSII}), and the coefficient of photochemical quenching (q_P), but increases the relative reduction state of PSII (Guo and Li, 2000). With long stress time, the chlorophyll content and the binding degree of chlorophyll protein complex decline gradually, the O_2^- (superoxide radical) production rate and the H_2O_2 content in leaves increase. Although the activities of superoxide dismutase, peroxidase, and catalase increase for 2–3 days under high-temperature stress, they decrease afterward. Nevertheless, the gradual increase of the superoxide dismutase, peroxidase, and catalase activities as well as that of the ratio of photorespiration rate (P_r) to $P_r + P_n$ and non-photochemical quenching of chlorophyll fluorescence suggest that those mechanisms related (NPQ) to the change of these parameters protect rice leaves from oxidative damage under high nocturnal temperature stress (Guo and Li, 2000). These studies show that enhancement of photosynthetic

capacity may become a prerequisite for greater yield potential in the future climatic conditions.

5.2. Respiration

Respiration is typically partitioned into growth respiration (the functional components of construction) and maintenance respiration (of maintenance and ion uptake) (Amthor, 1986; Lambers, 1985). Growth respiration is temperature dependent, only because it follows growth rate. But, the growth efficiency, which depends on the ratio of respiration and growth rate, may be independent of temperature. Increased respiration can lead to the production of reactive oxygen species, which can decrease membrane thermal stability. Maintenance respiration is mainly associated with turnover of proteins and lipids and maintenance of ion concentration gradients across membranes (Penning de Vries, 1975). It is very sensitive to environmental changes (Ryan, 1991) and strongly temperature dependent since it is directly related to the enzymatic processes of degradation. Toward the end of the crop cycle, leaf senescence will cause the decreased rates of leaf dark respiration.

Any increase in respiration in response to climate warming is of serious concern, as respiratory processes could consume a larger portion of total photosynthates (Paembonan *et al.*, 1992). High nighttime temperatures are generally considered to be disadvantageous because they can stimulate respiration (Zheng *et al.*, 2002). Mohammed and Tarpley (2009a) showed that there were no differences among the rice plants grown under high night temperature (32 °C) and ambient night temperature (27 °C) for leaf respiration rates at boot or mid-dough stage. However, at effective grain-filling stage, plants grown under both heat treatments (high nighttime temperature and ambient temperature) had 26% and 172% higher leaf respiration rates, compared with boot and mid-dough stages, respectively. In response to high-temperature stress, rice yield showed a negative association with leaf respiration rates and a positive association with leaf membrane stability (Mohammed and Tarpley, 2009b).

5.3. Enzymes

As biocatalysts, enzymes facilitate biochemical reactions by providing alternative lower activation energy pathways and thereby increasing the rate of reaction. To some extent, temperature increases enhance the rate of reactions. But, at very high temperatures, the loss of primary structure with associated covalent bond cleavage, which is irreversible, degrades many enzymes or some may get denatured by the loss of tertiary and secondary protein structures, not involving covalent bond cleavage, which is reversible. Starch in grains accounts for 90% of the total brown rice weight, and

the rice-grain-filling process is an important biochemical process, where sucrose hydrolyses and starch synthesis occur. Many enzymes are involved in the conversion of sucrose into starch in rice endosperm, including sucrose synthase [EC 1.9.3.1], starch synthase [EC 2.4.1.21], ADP-glucose pyrophosphorylase [EC 2.7.7.27], starch branching enzyme (SBE) [EC 2.4.1.18], and starch debranching enzyme [EC 3.2.1.70] (Kubo *et al.*, 1999). There are two groups of starch synthases: soluble starch synthase (SSS) [EC 2.4.1.21] and granule-bound starch synthase (GBSS) [EC 2.4.1.21], with several isoforms for each group (Ahmadi and Baker, 2001). The SSS is sensitive to temperature and its activity declines under heat stress, resulting in the reduction in rates of starch and amylase synthesis. Starch accumulation and composition in rice endosperms are under the coordinated regulation of several enzymes. Hirano and Sano (1998) reported a decrease in amylase concentration in *japonica* rice as a result of a decrease in granule-bound starch synthase activity. Hussain *et al*. (1999) have shown sucrose phosphate synthase is upregulated in rice grown under elevated [CO_2] and temperature. The activity and expression levels of soluble endosperm starch synthase were higher at 29/35 °C than that at 22/28 °C (Jiang *et al*., 2003). In contrast, the activities and expression levels of the rice branching enzyme, and the granule-bound starch synthase of the endosperm were lower at 29/35 °C than those at 22/28 °C, suggesting that the decreased activity of SBE reduces the branching frequency of the branches of amylopectin. Consequently, an increased amount of long chains of amylopectin occurs in endosperm at high temperature.

At high temperatures, the activities of ADP-glucose pyrophosphorylase and the concentration of sucrose increase, while starch accumulation and sucrose synthase activity decrease (Cheng *et al*., 2005). Although the granule-bound starch synthase is critical in controlling amylase concentration content in developing endosperms, other enzymes (starch debranching enzyme, SBE, ADP-glucose pyrophosphorylase, and starch phosphorylase) are responsible for cultivar differences in amylose accumulation at different temperatures.

In most plants, the production and accumulation of free and conjugated polyamines as well as increased activities of their biosynthetic enzymes have been associated with heat stress. Under 45 °C heat stress, the callus raised from heat tolerant and sensitive rice cultivars showed higher levels of free and conjugated polyamines as arginine carboxylase and polyamine oxidase activities were more in tolerant than in sensitive callus. Many uncommon polyamines, norspermidine, and norspermine were detected in the callus of the tolerant cv. N22 which increased appreciably during heat stress (Roy and Ghosh, 1996).

At higher temperatures, the maximal quantum yield of PSII photochemistry and the activity of ascorbate peroxidase increase (Han *et al*., 2009). The proteomics analysis of heat-stressed plants showed that proteins such as

lignification-related proteins are regulated and distinct proteins related to protection are upregulated at different high temperatures. In rice, different strategies are adopted at different levels of high temperature: at 35 °C, some protective mechanisms are activated to maintain the photosynthetic capability, while antioxidative pathways are active at 40 °C. At 45 °C, Hsps, in addition to those induced at 35 and 40 °C, are effectively induced in rice seedlings (Han *et al.*, 2009).

Rubisco [EC 4.1.1.39] catalyzes two competing reactions of RuBP carboxylation and oxygenation, the primary events in photosynthesis and photorespiration, respectively. The current atmospheric [CO_2] is insufficient to saturate RuBP carboxylases in C_3 plants. Any increase in the availability of this substrate results in a rise in leaf photosynthetic rates in the short-term measurements, partly high [CO_2] inhibits the oxygenation and the subsequent loss of CO_2 through photorespiration (Bowes, 1993). In addition to the atmospheric [CO_2], the photosynthetic rates of C_3 plants are affected by temperature, and this effect is also primarily exerted through rubisco (Long, 1991). Temperature strongly influences the [CO_2]-saturated photosynthesis greatly: increased temperature reduces the activation state of this enzyme, and decreases both the specificity for CO_2 and the solubility of CO_2, relative to O_2, resulting in greater losses of CO_2 to photorespiration as temperature rises. Consequently, a doubling of atmospheric [CO_2], and the concomitant inhibition of the rubisco oxygenase reaction, should moderate the adverse effects of high temperature on C_3 photosynthesis and result in even greater enhancement of net photosynthesis by elevated [CO_2] as growth temperatures increase (Long, 1991). Low temperature affects the rate of rubisco regeneration limited by electron transport and/or starch and sucrose synthesis to a greater relative extent than the rate limited by Rubisco capacity (Sharkey, 1985). Under high temperatures, photosynthesis at ambient [CO_2] is relatively limited by rubisco capacity. Vu *et al.* (1997) observed that CO_2 enrichment (twice ambient) and high growth temperatures (28–40 °C) reduced the Rubisco content of cv. IR72 by 22% and 23%, respectively. Fine control of rubisco activation was also influenced by both elevated [CO_2] and temperature.

Heat-induced changes of rubisco when estimated in tolerant (cv. N22) and sensitive (cv. IR8) cultivars of rice (Bose and Ghosh, 1995), a temperature of 40 °C increased specific activity of carboxylase and the titer of rubisco holoenzyme, estimated by preparing antisera, were increased or affected, and the specific activity and holoenzyme level were more stable in the tolerant cultivar than in the sensitive cv. IR8 at 45 °C. In both cultivars, a decline in activity and holoenzyme level with time was pronounced at 50 °C. Higher temperatures affect large subunit (RLSU) more than small subunit of rubisco (RSSU) in the tolerant cultivar. But, no such trend was noted in component proteins of the sensitive cultivar. The tolerant cultivar showed greater thermostability of the rubisco protein up

to 45 °C, whereas the susceptible cultivar (IR8) was thermolabile. The degradation of rubisco occurred in both the cultivars at 50 °C. There are genetic differences in rice cultivars for protective mechanisms against thermal degradation of rubisco (Bose *et al.*, 1999). In order to have rice with a positive acclimation to temperature changes, growth at high temperature should cause a relative increase in rubisco capacity and the growth at low temperature should promote RuBP regeneration capacity.

5.4. Carbohydrate accumulation and partitioning

Remobilization of carbohydrate from the leaf sheath and culm of rice to grain contributes to yield as much as 38% and the contribution varied considerably among rice varieties (Yoshida and Ahn, 1968). The leaf sheath plays a significant role in the temporary storage of starch. The steady-state mRNA levels of ADP-glucose pyrophosphorylase, SSS, and branching enzyme coincide with a rapid starch accumulation (Hirose *et al.*, 1999). Rowland-Bamford *et al.* (1996) determined the long-term effects of [CO_2] and temperature on carbohydrate partitioning and status in cv. IR30. The priority between partitioning of carbon into storage or into export in leaf blades changed with [CO_2] (330 or 660 μmol mol^{-1}) and temperatures (daytime air temperatures 28, 34, or 40 °C). At all temperatures, leaf sucrose concentration increased with CO_2 enrichment and elevated [CO_2] over the season resulted in an increase in total nonstructural carbohydrate concentration in leaf blades, leaf sheaths, and culms at all temperature treatments tested. Although elevated [CO_2] had no effect on carbohydrate concentration in the grain at maturity, total nonstructural carbohydrate concentration was significantly lowered by increasing temperature. Under the highest temperature regime, the plants in the 330 μmol mol^{-1} CO_2 treatments died during stem extension while the [CO_2]-enriched plants survived, but produced sterile panicles. The [CO_2]-enriched plants could survive and maintain carbohydrate production rates, with total nonstructural carbohydrate concentration not affected, at higher temperatures than the nonenriched plants (Rowland-Bamford *et al.*, 1996).

There is an early decline of assimilate storing ability of grains by high temperature during ripening period (Inaba and Sato, 1976). The respiration rate of grains declines with the progress of ripening, more rapidly at high temperature reaching the lowest level at 2 weeks after anthesis. The oxygen uptake by grain mitochondria follows a similar pattern as grain respiration and ADP/O ratio at high temperature reaches almost zero, whereas that at normal temperature remained fairly high until maturity. The water percentages of grains and leaf decrease rapidly with the progress of ripening, being always lower at high temperature. Inaba and Sato (1976) found that the carbohydrate and nitrogen contents of grains paralleled with 1000-grain weight during ripening at both temperatures, but protein-N at high

temperature did not increase, and nonprotein-N gradually increased thereafter. The phosphorylase activity reached a maximum, followed by a gradual decrease at high temperature. In addition, the succinic-dehydrogenase activity at rachilla disappeared, and soon yellowing started. A large amount of assimilate can occur in leaves and culms due to the occurrence of sterile spikelets, and consequently photosynthetic rate may be depressed due to the accumulation of starch in the chloroplasts in plants grown under high temperature (Oh-e *et al.*, 2007).

5.5. Heat shock proteins

Under supraoptimal temperature, there is a dramatic change in protein synthesis in living organisms, with reduction in the production of most proteins as well as the induction of a new set of proteins known as Hsps. Hsps are molecular chaperones, which function in protein folding and assembly, protein intracellular localization and secretion, and degradation of misfolded and truncated proteins. Heat shock factors (Hsfs) are the transcriptional activators of Hsps. Both Hsps and Hsfs are involved in response to various abiotic stresses such as heat, drought, salinity, and cold. The major classes of Hsps include Hsp100, Hsp90, Hsp70, Hsp60, and low molecular weight Hsps (also called sHsps). The proportions of the three classes differ among species. In general, Hsps are induced by heat stress at any stage of development. Under maximum heat stress conditions, Hsp70 and Hsp90 mRNAs can increase 10-fold and low molecular weight Hsp increase as much as 200-fold. In rice, heat-responsive gene profiling differed largely from those under cold/drought/salt stresses (Hu *et al.*, 2009). In the cells of callus derived from rice seed embryos, heat shock depresses normal protein synthesis, but enhances the synthesis of specific proteins (Fourré and Lhoest, 1989). Depending on whether the temperature increase is rapid or gradual, differences are observed in the production of Hsps.

The antibodies raised against yeast Hsp104 recognized a heat-inducible polypeptide with a molecular mass of 110 kDa in shoot tissue of young rice seedlings (Singla and Grover, 1993). Nevertheless, this polypeptide was seen to be constitutively present in the flag leaf of 90-day-old field-grown plant. Considering the crucial role of Hsp101 in imparting thermotolerance to cells, Katiyar-Agarwal *et al.* (2003) inserted the *Arabidopsis thaliana hsp101* (*Athsp101*) cDNA into cv. Pusa basmati 1 by *Agrobacterium*-mediated transformation and demonstrated the stable integration and expression of the transgene into rice genome. There was no adverse effect of overexpression of the transgene on overall growth and development of transformants, with the transgenic rice lines showing significantly better growth performance in the recovery phase following heat stress. Overexpression of Hsp101 can provide an advantage in thermotolerance in rice. Likewise, Murakami *et al.* (2004) found that transgenic rice plants (cv. Hoshinoyume) with increased

levels of sHsp17.7 protein, which is capable of protecting stressed catalase from precipitation, exhibited significantly increased thermotolerance compared to untransformed control plants. Lee *et al.* (2007) investigated rice leaf proteome in response to heat stress and found a group of low molecular small Hsps (sHsps) that were newly induced by heat stress. Among these sHsps, there was a low molecular weight mitochondrial (Mt) sHsp. In addition, they found that transcription levels were not completely concomitant with translation. Identification of some novel proteins in the heat stress response can provide new insights on molecular basis of heat sensitivity in rice plants.

5.6. Membrane injury

The cellular membranes, which regulate the flow of materials between cells and the environment as well as their internal compartments, are the critical sites of high-temperature stress. The membranes are the first structures involved in the perception and transmission of external stress signals. Adverse effects of temperature stress on the membranes include the disruption of cellular activity or death. Injury to membranes from a sudden heat stress event may result either from denaturation of the membrane proteins or from melting of membrane lipids, which leads to membrane rupture and loss of cellular content, and is measured by ion leakage. The membrane lipids are highly susceptible to changes in temperature and consequently changes in membrane fluidity, permeability, and cellular metabolic functions. Lipid saturation level typically increases, whereas unsaturated lipids decrease with increasing temperature. High temperature fluidizes by melting the lipid bilayer, increasing membrane permeability, and increasing leakage of ions and other cellular compounds from the cell. Modifications in membrane structure and composition play a key role in plant adaptation to high-temperature stress. In fact, maintaining proper membrane fluidity is essential for temperature stress tolerance. Mutants of soybean (*Glycine max* L.) that are deficient in fatty acid unsaturation maintained stable membrane fluidity and showed improved tolerance to high temperature (Alfonso *et al.*, 2001).

Increased cell damage as a result of high-temperature stress can decrease membrane thermostability, thereby disrupting water, ion, and organic solute movement across plant membranes, affecting all other metabolic activities (Christiansen, 1978). The membrane thermal stability, measured as the conductivity of electrolytes leaking from leaf disks at high temperature, is one of the simplest and best techniques to evaluate the performance of plants under high temperatures (Sullivan, 1972). In rice plants grown under high night temperature (32 °C), the membrane stability decreases (Mohammed and Tarpley, 2009b). Since the functional cell membrane system is central to crop yield productivity and adaptation of plants to high temperature, the leaky membranes can negatively affect crop productivity. Although Mohammed and Tarpley (2009b) considered the decreased

rice yields due to high temperatures as a result of leaf electrolytic leakage, Prasad *et al.* (2006) observed no relationship between electrolytic leakage and yield. Membrane lipid/fatty acid species and changes in lipid composition and fluidity that are important in regulation of thermotolerance in rice are unknown.

5.7. Pollen germination

Rice anthers dehisce at the time of floret opening and most of the florets, which are adichogamous, are self-pollinated. The driving force for anther dehiscence is the swelling of pollen grains at the time of floret opening (Matsui *et al.*, 1999). Temperature stress reduces the percentage of anthers dehiscing at the time of flowering (Shimazaki *et al.*, 1964). Pollination is sensitive to temperature: high temperatures at the time of flowering inhibit the swelling of pollen grains (Matsui *et al.*, 2000), whereas low temperatures at the booting stage impede pollen growth (Shimazaki *et al.*, 1964). High (>35 °C) and low (<20 °C) temperatures can result in poor pollination and loss of yield (Hori *et al.*, 1992). Changes in floral characteristics as affected by high-temperature conditions are provided in Table 2.

Interestingly, Satake and Yoshida (1978) observed that female fertility was unaffected by high temperature as seed setting was found in all the cases of hand-pollinated florets, except for those plants subjected to 41 °C. In addition, high temperature was found to cause anther dehiscence outside the spikelets in susceptible cultivars resulting in poor pollination. Shedding of a high number of pollen grains on stigma, even at high temperatures, was found to be a characteristic of tolerant cultivars such as cv. N22. Identification of sources of heat tolerance from the cultivars has led to programs where breeding lines are subjected to high temperature in the phytotrons (IRRI, 1978, 1979).

Yamada *et al.* (1955) made observations on the effect of different temperatures on the pollen germination of cv. Kyoto-asahi on artificial media and found that the maximum and minimum temperatures for germination were 42–45 and 12–15 °C, respectively. In another study using 14 Japanese (6 early, 4 medium, and 4 late cultivars) and 9 foreign cultivars, Enomoto *et al.* (1956) found that the maximum temperature limits were 40–45 °C and the minimum limits were 7–14 and 10–14 °C for the Japanese cultivars. During flowering, high temperatures, even for only a few hours, can cause significant reduction in floral reproduction (Matsushima *et al.*, 1982; Satake and Yoshida, 1978; Sato *et al.*, 1973). Sterility in heat-sensitive cultivars is chiefly due to a reduction in the number of deposited pollen grains on a stigma although female sterility can also occur at higher temperatures (>40 °C) (Satake and Yoshida, 1978). More than 10 germinated pollen grains are required for normal fertilization of rice. High temperature sterility is due to a drop in the number of germinated pollen on a stigma to less than

nine (Satake and Yoshida, 1978). Poor germination of pollen grains may also be a cause of sterility (Matsui *et al.*, 1997b) under field conditions.

When the amount of pollen shedding on stigma was studied by Mackill *et al.* (1982) under the two temperature regimes (29/21 and 38/27 °C), the tolerant cultivars were found to have more pollen grain shedding on stigma under both temperature regimes. In contrast, there was a marked reduction in the amount of pollen shed on stigma at high temperature in the susceptible genotypes. The percentage fertility was found to be positively correlated with the amount of pollen on stigma at 38/27 °C. The large amount of pollen on stigma in tolerant genotypes appeared to compensate for reduced pollen grains grown under high temperature. In cv. IET4658, the pollen germination was reduced under the 38/27 °C temperature regime.

High temperature changes some of the traits of reproductive organs such as increasing anther pore size and reducing stigma length, and pollen number, and anther protein expression (Jagadish *et al.*, 2010). Although the number of pollen on the stigma was not related to anther length and width, apical and basal pore lengths, apical pore area, and stigma and pistil length, the variation in spikelet fertility was highly correlated with the proportion of spikelets with more than 20 germinated pollen grains on the stigma. The analysis of anther protein expression by a 2D-gel electrophoresis suggested that there were about 46 protein spots changing in abundance, of which 13 differentially expressed in both tolerant and susceptible genotypes. In the tolerant cv. N22, there was an upregulation of a cold and a heat shock protein, probably contributing to the heat tolerance.

5.8. Spikelet sterility

Rice can be grown vegetatively with daytime temperatures as high as 40 °C, but floral development is very sensitive to high temperatures. The susceptibility to high-temperature-induced floret sterility is highest at flowering stage, followed by booting stage (Satake and Yoshida, 1978). Osada *et al.* (1973) reported that temperature exceeding 34–35 °C resulted in a high percentage of sterile spikelets in Thailand. Similar threshold was observed in *japonica* rice cultivars under controlled conditions (Horie *et al.*, 1995a) and those by Matsui *et al.* (1997a,b). It is likely that the predicted global warming increases the occurrence of high-temperature-induced floret sterility in rice (Matsui, 2009). Temperatures at which sterility occurs vary with the cultivars: temperature above 35 °C during anthesis can result in 90% floral sterility in several rice cultivars (De Datta, 1981). Poor anther dehiscence, decrease in the number of pollen grains on the stigma, and poor germination of pollen on the stigma are the principal causes of sterility (Satake and Yoshida, 1978; Imaki *et al.*, 1983; Matsui *et al.*, 1997a, 2001). Spikelet fertility increases linearly with the number of germinated pollen grains per stigma (Matsui *et al.*, 1997b).

Many growth chamber studies unequivocally show high-temperature-induced sterility (Matsushima *et al.*, 1964c; Sato, 1979). Probably, Satake and Yoshida (1978) provided the most detailed and precise information using the phytotron facilities at the IRRI (Philippines) and showed that anthesis was the most sensitive stage to high temperature in three *indica* (tropical) rice selections (N22, IR747B2-6, and BKN6624-46-2). The flowering of spikelets, immediately before or after high temperature, was not affected. Increase in temperature from 35 to 41 °C as well as the duration of temperature treatment increased the percentage sterility. But, the night temperatures between 21 and 30 °C did not affect spikelet fertility, but a night temperature of 33 °C was found to decrease fertility.

The critical temperatures to induce 50% sterility were about 36.5 °C for cv. Akhihikari and 38.5 °C for cv. Koshihikari when these two *japonica* cultivars were treated for a 6-h high-temperature treatment of panicles for 8 days at flowering. Matsui *et al.* (1997a) attributed the major cause for the difference between the two cultivars to differences in the number of pollen grains shed on the stigma. In another study using open-top chambers (OTCs) in field under combinations of ambient [CO_2], temperature, +4 °C, and +300 μmol mol^{-1}, Matsui *et al.* (1997b) observed that high temperature during flowering resulted in increased pollen sterility with the degree of sterility exacerbated if cv. IR72 was exposed to both temperature and increased [CO_2]. The critical air temperature for spikelet sterility (as determined from the number of germinated pollen grains on stigma) is reduced by 1 °C at elevated [CO_2], suggesting that the downward shift in critical temperature may be due to the observed increase in canopy temperature at high [CO_2]. This increase in canopy temperature, in turn, may be related to partial stomatal closure and reduced transpirational cooling in an elevated [CO_2] environment.

In general, all rice genotypes are not considered suitable for cultivation in any particular season. Therefore, selection of cultivars for the predicted future climate will be a daunting task. From a study using 14 rice cultivars of different species (*Oryza sativa* and *Oryza glaberrima*), ecotypes (*indica* and *japonica*) and origin (temperate and tropical) exposed to ambient and high temperature (ambient and +5.8 °C) at Gainesville, Florida, Prasad *et al.* (2006) observed that high temperature significantly decreased spikelet fertility across all the selected cultivars, but effects varied among cultivars. Tolerance or susceptibility is not species- or ecotype dependent as some cultivars in each species or within ecotypes of tropical and temperature origin are equally susceptible to high temperature. Decreased pollen production and pollen reception (pollen numbers on stigma) are some of the main causes for decreased spikelet fertility, leading to fewer filled grains, lower grain weight per panicle, and decreased harvest index, and cultivar difference. Prasad *et al.* (2006) suggested that spikelet fertility at high temperature can be used as a screening tool for heat tolerance during the

reproductive phase. After being grown at 30/24 °C day/night temperature in a greenhouse and transferred to growth cabinets for the temperature treatments [29.6 (control), 33.7, and 36.2 °C tissue temperatures], the pattern of flowering in cultivars IR64 (lowland *indica*) and Azucena (upland *japonica*) was found to be similar, peak anthesis occurred between 10:30 and 11:30 h at 29.2 °C, and about 45 min earlier at 36.2 °C (Jagadish *et al.*, 2007). In both genotypes $\geq$1 h exposure to $\geq$33.7 °C at anthesis caused sterility. In cv. IR64, there was no interaction between temperature and duration of exposure, and spikelet fertility was reduced by about 7% per 1 °C over 29.6 °C. In contrast, there was a significant interaction between temperature and duration of exposure, and spikelet fertility was reduced by 2.4% per 1 °C per day above a threshold of 33 °C in Azucena. Jagadish *et al.* (2007) considered marking individual spikelets as an effective method to phenotype the genotypes and lines for heat tolerance that removes any apparent tolerance due to temporal escape.

Rice spikelets typically flower during late morning with peak anthesis occurring between 10:00 and 12:00 h. Many genotypes have been screened for tolerance to high temperature during flowering (Satake and Yoshida, 1978; Matsui *et al.*, 2001; Matsui and Omasa, 2002; Prasad *et al.*, 2006) at temperatures up to 41 °C and for durations ranging from 2 h to the whole crop cycle. A positive correlation exists between the sterile spikelets and the maximum temperature during the flowering period (first heading to full heading), and the percentage of sterile spikelets exceeds 10% when the maximum temperature is around 37 °C (Oh-e *et al.*, 2007). The time of flowering of rice differs among cultivars, with some cultivars flowering early in the morning, and such cultivars are useful to avoid damage by high temperatures at the flowering time (Imaki *et al.*, 1983).

The spikelet tissue temperature of 33.7 °C even for an hour at anthesis induces spikelet sterility (Jagadish *et al.*, 2007). But, temperatures of 38 and 41 °C at an hour before or after anthesis do not affect spikelet fertility (Yoshida *et al.*, 1981). Exposure to high temperature (centered on the time of peak anthesis) and duration (more than 2 h) reduces spikelet fertility and genotypic ranking is highly correlated, suggesting a consistent and reproducible response of spikelet fertility to temperature (Jagadish *et al.*, 2008).

5.9. Grain filling

Temperature is one of the most important environmental factors governing grain filling. Because of environmental fluctuations, temperatures are often higher than optimum, thus increasing the probability of the grain being exposed to extended periods of supraoptimal temperatures during crop growth in many rice-producing areas. Such temperatures are detrimental for rice grain filling (Tashiro and Wardlaw, 1991a,b; Figs. 2 and 3). The head

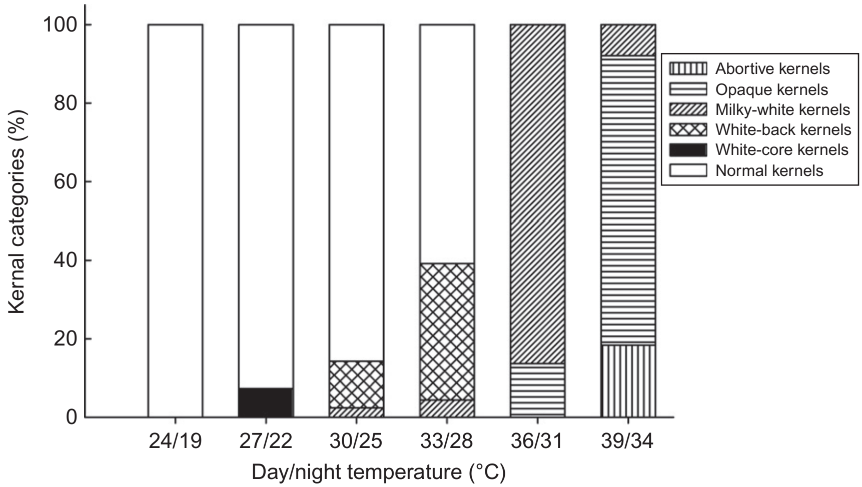


Figure 2 Effect of temperature on kernel damage on rice. The temperature treatments were imposed 7 days after heading and continued to maturity (adapted from Tashiro and Wardlaw, 1991a).

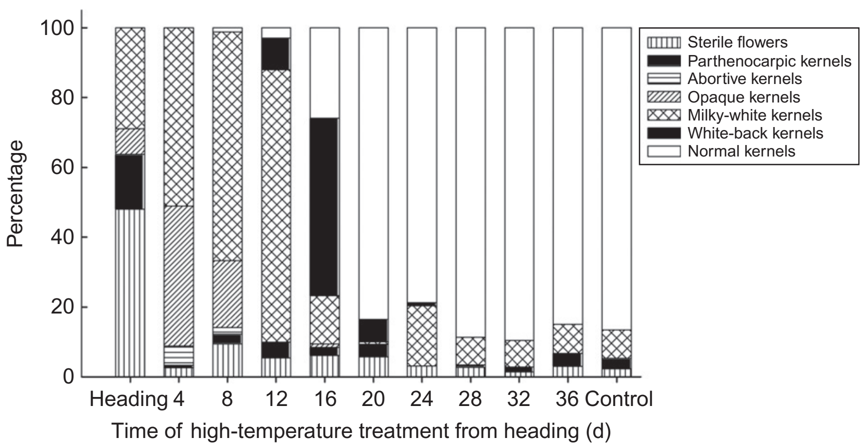


Figure 3 Effect of high temperature (36/31 °C), for a period of 8 days at intervals of 4 days commencing at heading, on kernel damage in the fourth and fifth spikelets from the apex of the central four primary branches of a panicle of rice at maturity (adapted from Tashiro and Wardlaw, 1991a).

rice yield is related to the cellular structure of the starch containing molecules within rice grains, and this structure is temperature sensitive.

Individual grains within a panicle show considerable variation but the overall grain weight is a stable cultivar characteristic of rice. There exists

apical dominance during grain filling, and the delayed filling of inferior spikelets results from source limitation and regulation of assimilate allocation within the panicle. Adequate translocation of assimilates to all the spikelets in a panicle can increase grain yield. Even a brief exposure to high temperatures during seed filling can accelerate senescence, diminish seed set and seed weight, and reduce yield (Krishnan and Surya Rao, 2005; Siddique *et al.*, 1999).

High-temperature injuries are due to disappearance of enzyme activity relating to starch synthesis of the grains. The rice grain grown at 38/21 °C contains more chalky grains, a characteristics influenced by shape, size, and packing of amyloplasts in kernels, which are different from those in translucent grains (Lisle *et al.*, 2000). Compared with a high day temperature (34/22 °C, day/night), high night temperature (22/34 °C, day/night) causes a reduction in final grain weight and growth rate of rice in the early and mid stages of grain filling, along with a reduction of final grain weight and growth rate of cells (Morita *et al.*, 2005). Moderate, cool temperatures often benefit grain yield because lower temperatures reduce the growth rate of grain, extending the duration of the grain-filling period, and delaying grain maturation (Shimono *et al.*, 2002; Yoshida, 1981). Along with dehydration of water, numerous biochemical and physiological changes occur in tissues during seed maturation. The increase in the amount of chalk grains due to high temperature causes grains to break during polishing, lowering the amount of rice for consumption. During grain-filling stage, high temperature significantly shortens assimilate supply time (Fitzgerald and Resurreccion, 2009). There are differences among cultivars in regulation of substrate supply, architecture of the panicles, and the capacity of the panicles to alter sink size in response to heat stress, which manifest in differences in edible rice.

There are significant decreases in grain dry weight with increases in temperatures during the period of grain development (Tashiro and Wardlaw, 1991a). The greatest change in dry weight of the grains takes place when heat stress in grains occurs during the linear phase of dry matter accumulation. Interestingly, the flow of nitrogen into grains is more stable than that of carbon as temperatures are increased. High temperatures interfere with the early stages of cell division and development in the endosperm. Grain thickness is reduced most by high temperature on day 12 after heading; length and width of grains are affected when high temperatures occur earlier in development. Abortive and opaque grains are numerous when high temperature commenced 4 days after heading (Tashiro and Wardlaw, 1991b). Depending on both the temperature level and duration, chalky endosperm tissue occurs in several forms: white-core kernels are evident at a temperature of 27/22 °C, and white-back kernels are most numerous at 36/31 °C when high-temperature stress occurs 16 days after heading. The development of numerous air spaces between loosely packed

starch granules and a change in light refraction are the major causes of the chalky appearance.

Extreme high day temperatures during the grain-filling period may reduce starch synthesis in the grains and, especially so under N-deficient conditions (Ito *et al.*, 2009). High temperatures also induce an accumulation of sucrose and a decrease in carbon and nitrogen transport from the shoots to the ears via the phloem. The enzymatic activity of starch synthesis is closely related to the formation and filling of grains (Jeng *et al.*, 2003). Shortening of the ripening period in rice due to high temperature is caused by higher activity of enzymes involved in starch synthesis during the early grain growth stage (Oh-e *et al.*, 2007). When different expressions of three isoform genes (*SBEI*, *SBEIII*, and *SBEIV*) encoding SBE in the endosperms were studied by real-time fluorescence quantitative polymerase chain reaction (FQ-PCR) method, Wei *et al.* (2009b) found that the effects of high temperature on the SBE expression in developing rice endosperms are isoform dependent. High temperature significantly influences the isoform expression, downregulating the expressions of *SBEI* and *SBEIII*, while upregulating the expression of *SBEIV*. Compared with *SBEIV* and *SBEIII*, the expression of *SBEI* gene in rice (cv. Zhefu 49) endosperms is more sensitive to temperature increase at the grain-filling stage. The ATPase activity in grains is significantly reduced, especially in the heat-sensitive genotypes, but with slight influences in the heat-tolerant genotypes (Cao *et al.*, 2009). High temperatures during the grain-filling period increase the rate of grain dry matter increase as a sink capacity, but this increase is insufficient to completely compensate for the concomitant reduced filling period. Probably, the failure of assimilate supply to the grain to meet the requirements of the accelerated grain dry matter increase leads to yield reductions under high temperatures. During the last half-decade, the rising temperature has affected rice quality in western Japan (Kobata *et al.*, 2004). Lack of assimilate supply to grains is hypothesized to increase the proportion of milky white rice grains, because high temperatures during the grain-filling period could increase the grain growth rate.

The extent of damage caused by high-temperature stress depends on the time of exposure in relation to the stage of grain development (Sato *et al.*, 1973; Zakaria *et al.*, 2002; Ito *et al.*, 2009). By exposing the rice panicles to high-temperature stress during 7 days after heading, cell division and ultimately the number of endosperm cells and starch granules are severely reduced (Funaba *et al.*, 2006), which was associated with increased spaces among the amyloplasts (Zakaria *et al.*, 2002). In rice under high-temperature stress, high chalkiness and poor edible quality are closely related with starch synthesis in endosperm during grain filling (Umemoto and Terashima, 2002; Jin *et al.*, 2005). Even when rice panicles are exposed to heat stress at later developmental stages (e.g., the linear filling period), there

is a significant repression in starch biosynthesis because of the reduction in the activity of these enzyme (Kobata and Uemuki (2004). Under high-temperature stress, the expression of *SBE* genes as well as the expression difference of each isoform gene during grain filling may determine the structure of starch in rice endosperm and the quality of rice grains (Wei *et al*., 2009b).

6. Effects of High Nighttime Temperature

Environmental temperature, especially nighttime temperature during grain development, plays an integral role in grain quality (Cooper *et al*., 2008) and is difficult to predict. Its influence can only be manipulated to some extent with the choice of planting dates. Many historical analyses have indicated that decreased yields are often correlated with increased nighttime temperature during the growing season (Downey and Wells, 1975; Peng *et al*., 2004). High nighttime temperatures are related to decreased panicle mass (Ziska and Manalo, 1996) and increased numbers of chalky kernels (Yoshida and Hara, 1977). Yoshida and Hara (1977) noted that kernel dimensions decreased with increased nighttime temperature. The head rice yield is influenced by the thickness distribution pattern of a population of rice kernels and, by altering the thickness distribution of kernels, an increase in nighttime temperature could potentially reduce head rice yield (Sun and Siebenmorgen, 1993; Siebenmorgen and Cooper, 2006). In general, as nighttime temperature increases, head rice yield decreases (Counce *et al*., 2005). High nighttime temperatures during grain development can cause an increase in amylose content (Resurreccion *et al*., 1977), and the proportion of long chains of amylopectin can decrease (Counce *et al*., 2005). The head rice yield can be related to the cellular structure of the starch containing molecules within rice kernels, and this structure is temperature-sensitive.

7. Interaction Between Humidity and High Temperature on Rice

The effects of temperature on rice may be intermingled with those of RH and solar radiation. The mean RH during rice cultivation is generally negatively associated with solar radiation. Among *japonica* cultivars, there are cultivar differences in the effects of both high temperature and high humidity on spikelet fertility (Morokuma and Yasuda, 2004). High humidity of 88% at 35 °C decreases fertility percentages, and the degree of decline

differs among the cultivars. Under high humidity at 31 °C, pollination is cultivar dependent, but not fertility percentage. High humidity increases the percentage of spikelets with only a few pollen grains on the stigmas and thereby lowers fertility.

Spikelet sterility at high air temperatures increases with increased humidity (Nishiyama and Satake, 1981; Matsui *et al.*, 1997b). Similarly, low humidity can promote spikelet sterility under high temperature, as shown by Matsushima *et al.* (1982) in an experiment of rice cultivation in Sudan. Matsui *et al.* (1997b) showed that fertility of spikelets at 37.5 °C was highest at 45% RH followed by that at 60% RH and lowest at 80% RH. Low humidity at high temperature disturbed the pollen shedding and decreased the number of germinated pollen grains on the stigma. Almost complete grain sterility in rice could be induced by 35 °C day and 30 °C night air temperature when coupled with 85–90% RH at heading (Abeysiriwardena *et al.*, 2002). In tropical ecosystems, high-temperature-induced grain sterility in rice is already a serious problem. Under high-temperature stress at flowering, fertility of rice cultivars is affected. Dry air due to low humidity promotes dehiscence of anthers or curbs extra elongation of filaments under high-temperature conditions (Nishiyama and Satake, 1981).

Increasing both air temperature and RH significantly increases spikelet sterility, while decreasing RH decreases the high-temperature-induced sterility (Weerakoon *et al.*, 2008). Increased spikelet sterility is generally due to increased pollen grain sterility which reduces deposition of viable pollen grains on stigma. With decreased RH, the reduction in sterility is more due to decreased spikelet temperature than due to air temperature. With spikelet fertility being linearly related to spikelet temperature, grain sterility increases when spikelet temperature increases over 30 °C and becomes completely sterile at 36 °C.

The temperature difference (TD) between the air and organs of rice plant varies with air temperature, air humidity, and plant type (Yan *et al.*, 2008). For similar air humidity, TDs were found to be lower at the air temperature of 28.5 °C than at higher temperature of 35.5 °C, whereas for the same air temperature, the TDs decreased as the air humidity increased. Moreover, the TDs were affected by cultivar plant type: erect panicle cultivars show higher TDs than those with droopy panicles under similar climatic conditions, and cultivars with panicles above flag leaf had higher TDs than those with panicles below the flag leaf. Yan *et al.* (2008) observed that the cultivars grown in a location with lower air humidity and higher temperature, such as Taoyuan, China, had higher spikelet fertility than those in higher humidity under the similar air temperature during the grain-filling stage, partially attributing this difference to the larger TDs under the lower humidity.

8. Effect of Changes in Temperature of Floodwater and Soil on Rice

Rice is grown on alluvial plains, flooded valleys, and terraced hillsides, suggesting an equally wide variety of soils on which rice can be cultivated. The most important soil orders are Alfisols, Entisols, Inceptisols, and Ultisols, while other orders can be significant at certain rice-growing areas (Moorman and van Breemen, 1978; Neue *et al.*, 1990). Flooded conditions of many rice soils are not natural, but are induced by man and the physical conditions that permit 10–20 mm water day^{-1} are necessary for high yields (Ponnamperuma, 1972). The generalized model of nutrient cycling in submerged rice cultivation is presented in Fig. 4 with possible changes under high-temperature conditions. The fertility of soils depends on the influences of soil and water temperature as the inherent fertility and availability of plant nutrients in rice soils become reliant on the nature of mineralogical parent materials and on the degree of weathering, mediated by the edaphic and microbial processes. Although fertilizers can, to some extent, supplement low fertility levels, they are not widely applied in many

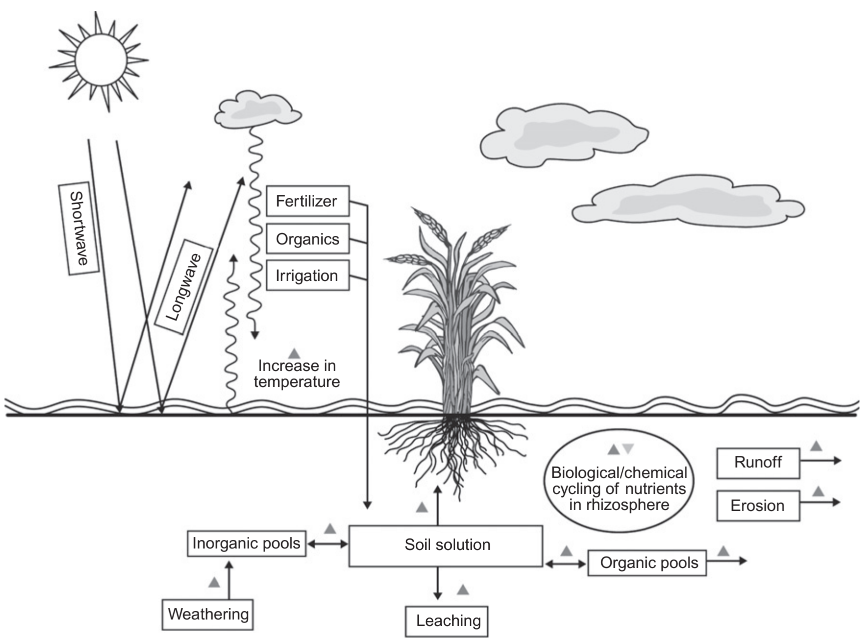


Figure 4 A generalized model of nutrient cycling in submerged rice cultivation under high-temperature condition.

rice-growing areas, owing to economic constraints (Pingali *et al.*, 1997). Flooded rice soils use relatively little herbicides and cause very little nitrate pollution of groundwater. But, they can produce more of the greenhouse gas methane, but less nitrous oxide. Information on the effects of soil temperature on the rates of chemical reactions, the physiological aspects of ion uptake, and the structure and function of the microbial communities in rice soils are scanty.

8.1. Influences of floodwater and temperature

The floodwater temperature is determined by many factors that include the balance of energy input and output in soil and can change continuously on a diurnal and seasonal basis. In the case of rice under flooded conditions, the temperature and the flow velocity of irrigation water influence the plant temperature which is regulated by various factors including solar radiation, cloud cover, wind speed, solar heat flux, and the transpiration of plants. The response of rice yield to soil water status varies with growth stage, being most sensitive at flowering, followed by the booting and the grain-filling stages (O'Toole, 1982). When the growing points of leaves, tillers, and panicles are under water, the temperature of water affects rice growth more than air temperature (Tsunoda and Matsushima, 1962). Floodwater interferes with gas exchange and light interception. Since the resistance to gas diffusion in water is 10,000 times more than that in air, restricted diffusion of oxygen and carbon dioxide is one of the limiting factors for plant survival and growth. In addition, the presence of algal growth or high water turbidity leads to poor light transmission. At different growth stages, the crop growth rate and leaf photosynthesis are influenced by the floodwater temperature (Shimono *et al.*, 2002, 2004). In another report, Ohta and Kimura (2007) showed that the floodwater temperature during the growing season for the future climate (2081–2100) would increase by approximately 1.6–2.0 °C throughout Japan, causing a northward shift of the isochrones of safe transplanting dates for rice seedlings. It is likely that one-fifth of current total cultivation of Japan area will be affected by high-temperature stress in rice plants. Floodwater temperature will change the respiratory costs since the rates of both anaerobic and aerobic metabolism are affected by temperature. The floodwater temperature is important for the influence on the temperature-dependent soil biochemical transformations and on the nutrient availability (Chaudhary and Ghildyal, 1970; Zia *et al.*, 1994). Since the floodwater temperature is affected by the partition of solar energy between air, water, and soil, the expected increases in temperature will disturb the energy balance in flooded rice fields.

When grown in flooded soils with varying water depths, the floodwater temperature affects rice growth when the growing point is in water (Tsunoda and Matsushima, 1962). Until the initiation of panicle primordia, the growing points of leaves, tillers, and panicles are under water, and water

temperature affects growth and development. Nevertheless, the leaf elongation and plant height growth can be affected by both air and water temperatures. Both the magnitude of temperature and water depth determine the effects of floodwater temperature on rice plants. Usually, water temperature is higher than air temperature, and increasing the water depth extends the duration during which water temperature controls growth (Yoshida, 1981). In spring and early summer of rice in mid and high latitudes, the thermal mitigation provided by water layer is significant against the climatic risk of low temperatures (Confalonieri *et al.*, 2005). Khakwani *et al.* (2005) observed that under high temperature (up to 41 °C) young seedlings can stand well in shallow water. Actually, an efficient method of protecting rice plants against sterility caused by low air temperature is to increase the water depth about 15–20 cm at the reduction division stage (Nishiyama *et al.*, 1969).

Variation does occur on the size of leaves as well as the number and diameters of the crown roots in rice plant, when subjected to high water temperature (35 °C) at the different developmental stages of the leaf and crown roots primordial (Sasaki, 1992). High water temperature decreases both the length and the width of leaf blade, but not the sheath length. High water temperature at each stage before the emergence of the crown root decreases the total number of crown roots emerged, except at the stage of initiation of crown root which increases in their number. At the stage before the initiation of the crown root primordia, high water temperature decreases the diameter of both the upper and lower roots.

Even short periods of high water temperature (35 °C) affected the growth response of immature leaves, with notable blade restraints, in rice plants (Sasaki, 2002). As the growing panicles reach above the water surface around reduction division stage, air temperature becomes dominant in controlling panicle growth and ripening (Tsunoda and Matsushima, 1962; Matsushima *et al.*, 1964b). The effects of air or water temperatures on grain yield and yield components may vary with growth stage: water temperature affects yield by affecting the panicle number per plant, spikelet number per panicle, and the percentage of ripened grains at early growth stages and air temperatures affect yield by affecting the percentages of unfertilized spikelets and percentages of ripened grains at later growth stages (Matsushima *et al.*, 1964a,b). High floodwater temperature retards rice growth, and seedlings grown in floodwater which is constantly above 38 °C die within few days after transplanting. Grain yield per hill decreases sharply with an increase in average daytime temperature of irrigation water from about 27 to 34 °C (Yoshida *et al.*, 1981).

8.2. High-temperature effects on submerged soil processes

The net amount of radiation reaching the soil surface which is a function of latitude and season determines the soil thermal regime. Altitude also affects soil temperature, with low-elevation soils warming more and earlier in the

spring than those higher up. The thermal conductivity of most mineral components of the solid phase of soils is similar; differences in thermal conductivities of mineral soils are due to water content and bulk density. Since the specific heat of water (1.0 cal g^{-1}) is greater than that of soil minerals (~0.2 cal g^{-1}), the moisture content greatly influences thermal capacity and diffusivity (Pregitzer and King, 2005). The prevailing climate is the major determinant of diurnal and seasonal progression of soil temperature. Land-use practices, plant cover, cultivation of soil, and moisture status are other factors which influence soil temperature significantly. The soil management practices for rice include flooding, puddling, maintaining a layer of standing water while the crop is on the land, draining and drying the fields, and reflooding for the next rice crop. The water level in rice fields often varies from 2.5 to 15.0 cm depending on the availability of water and the type of management practices followed. The flooding of soils leads to cutting off the oxygen supply because of the low solubility and diffusion of oxygen in floodwater. Within a short period of flooding, aerobic microorganisms utilize the available oxygen and render the bulk soil virtually free of molecular oxygen. When partial pressure of oxygen decreases, carbon dioxide concentration generally increases in soil environment. Patrick (1981) reported that anaerobiosis plays a significant role in these soils because of its multiple effects on the soil environment such as toxicity of anaerobic compounds, solublization of trace elements, and biological transformations. Alcohol formation and ethylene production have certain adverse effects on plants too (Ponnamperuma, 1965). In soils high in active iron but low in other nutrients, iron toxicity to rice plants is common. In soils with a thermic or hyperthermic temperature regime, the accelerated rates of mineral weathering and decomposition can increase the content of low-activity clays and decrease organic matter.

A flooded rice field functions like a greenhouse, where the layer of water acts like the glass of a greenhouse (Halwart and Gupta, 2004). The shortwave radiation from the Sun heats up the water column and the soil layer, but longwave radiation is blocked from escaping, thus raising the temperature of water and soil layers. During the daytime, solar radiation is absorbed at the surface, and heat energy is transferred to the overlying water by convection and to the underlying soil by conduction (Mowjood *et al.*, 1997) so that the soil temperature becomes relatively cooler than when there is no overlying water. Maximum temperature measured at the soil/water interface can reach 36–40 °C during mid-afternoon, sometimes exceeding 40 °C during the beginning of the crop cycle. Diurnal fluctuations can be about 5–16 °C, decreasing with the increased density of the rice canopy. Water has a specific heat capacity that is five times greater than dry soil, making flooded fields warming much slower and giving up its heat slowly as well. The temperature of floodwater can influence the phytoplankton productivity and photosynthesis, and may have a species-selective

effect; higher temperatures favor the cyanobacteria, while lower temperatures stimulate the eukaryotic algae.

What is currently not known is how the changes in the rates of soil reduction processes due to high soil or water temperature affect nutrient availability to rice plants. Due to either increases in denitrification and nitrate reduction or decreases in effective N concentration due to altered residue decomposition, the available soil nitrogen may decline. The reduction of various inorganic redox systems are carried out by different types of microorganisms (Watanabe and Furusaka, 1980; Sethunathan *et al.*, 1983; Ramakrishnan *et al.*, 2001). The more difficult is the reduction, the fewer the species that will carry out the reduction reaction. An important role of the inorganic redox systems in flooded soils is to support organic matter decomposition. The decomposition of organic matter supported by the nitrate, manganese, and iron systems is similar to the decomposition supported by the oxygen since the carbon dioxide and the reduced oxidant are the major products of this type of decomposition. Increasing temperature accelerates organic matter decomposition and decreases redox potential (Tsutsuki and Ponnamperuma, 1987), which can increase the rates of methane production greatly in flooded rice soils. Parashar *et al.* (1993) observed a distinct increase in methane emission from rice plots with increase in soil temperature from 26 to 34.5 °C. Chin and Conrad (1995) reported changes in the degradation pathway of organic matter and community structures of methanogenic archaea with a shift in the incubation temperature of rice soil from 30 to 15 °C, resulted in decreases in methane production. High temperature coefficients for methanogenesis were observed for paddy soils (Tsutsuki and Ponnamperuma, 1987; Rath *et al.*, 2002). Organic matter decomposition within tropical wetland rice soils can proceed as fast as under aerated dryland conditions due to many factors which include shallow floodwater and soil temperature of 30–35 °C. The mineralization of organic carbon is expected to be high at elevated temperatures (White *et al.*, 2000) and consequently, tropical soils will contain less organic carbon than temperate soils. Due to global warming, elevated temperature can lead to increased methane emissions not only in tropical soils but also in temperate soils. Neue *et al.* (1997) opined that small differences in climate, water, and nutrient regimes can change the delicate balance of wetland rice agriculture. Under flooded conditions, phenolic compounds which can affect the availability of soil nitrogen for plants may accumulate in soils (Unger *et al.*, 2009). There are reports that elevated temperatures manipulated with different warming facilities in the field stimulate net N mineralization rate in various biomes across the world (Rustad *et al.*, 2001). Water availability controls soil microbial activity, and as the controlling factors over soil microorganisms, the interactions of temperature and moisture can affect net nitrification/denitrification and mineralization rates in certain soils (Wang *et al.*, 2006). Compared to the

literature available on air temperature on rice plants, little is known regarding the influence of soil or floodwater temperature on nutrient transformation processes and their effects on rice plant nutrition and on the sustainability of flooded rice cultivation.

9. Simulation Modeling Studies on High-Temperature Stress on Rice Crop

The predictions and projections on future climate change are mainly obtained from the simulation studies using number of global climate models, each with different mathematical representations of the climate system and different capabilities. The simulation models are applied in plant sciences too and are one of the analytical as well as decision-making tools. Crop growth models help to understand the complex interactions among different environmental variables that influence growth and yields of crops (Krishnan *et al.*, 2008). The process-based crop simulation models that predict growth, development, and yield of crops use various inputs such as the local environmental conditions including weather and soil physical and chemical characteristics, crop management, and genetic information. These crop simulation models can be employed to determine the short-term impact of weather on growth and development as well as the long-term impact of climate and associated environmental risks on crop yield (Matthews and Stephens, 2002; Krishnan *et al.*, 2007). The mechanistic models of crop growth help to assess the effects of environmental variables that are often correlated with each other on crop yields (Sheehy *et al.*, 2006).

As early as 1980s, the MACROS crop simulation model was used to study the effect of climate change on rice production at the IRRI, Philippines (Penning de Vries *et al.*, 1989). Using the weather data from four contrasting sites (the Netherlands, Israel, the Philippines, and India), simulation on the average grain yield and its variability of rice under both fully irrigated and rain-fed conditions was performed. By using the MACROS crop simulation model, Pening de Vries *et al.* (1989) suggested rice yield increases of 10–15% due to a doubling of the CO_2 level, but the effect of the expected accompanying rise in temperatures would offset those increases. Increased photosynthesis at higher CO_2 levels, and reduced length of the growing season and increased maintenance respiration rates at higher temperatures were the plausible changes in the physiological activities under elevated [CO_2] and high temperatures. Describing the relationship between yield and minimum temperature over the range 22.1–23.7 °C using a quadratic equation, Peng *et al.* (2004) suggested that yield declined with minimum temperature by 10% per 1 °C and yield declined with average temperature by 15% per 1 °C (given the relative contributions of maximum

and minimum temperatures to mean daily temperature). Much smaller yield changes with temperature ranging from about 2% to 6% per 1 °C from a base yield for the temperature range 22–32 °C were suggested by other workers (Saseendran *et al.*, 2000). Simulation models were used to study the effect of high temperature on seed-setting rate and grain yield by combining the daily flower characteristics (Challinor *et al.*, 2005).

Using ORYZA 2000 rice model and after separating the effects of other environmental factors from high temperature, Sheehy *et al.* (2006) suggested that crop responses to temperature (below the high temperatures that cause infertility in rice) were of the order of -0.5 Mg ha^{-1} per 1 °C (or about -6% per 1 °C at the base yield at average mean daily temperature of 26 °C). Generally, the minimum temperature is not used for the simulation of any processes in rice simulation models. There is a strong need to develop new generations of crop models for rice as some of the present models are based on regression from selected weather elements, which can mislead because of correlations among the weather elements (Sheehy *et al.*, 2006).

Shi *et al.* (2007) have developed a process-based model to simulate the high-temperature-induced sterility, which considered the flowering characteristics of rice and daily change of air temperature. Hypothesis that high temperature induces spikelet injury was evaluated by Krishnan *et al.* (2007) by enhancing the tolerance level of cv. IR36 in the ORYZA1 model. Without any temperature tolerance of cultivar, large decreases in yield due to spikelet sterility were predicted. But, through the adaptation of cultivar with improved temperature tolerance, the grain yield increased by about $+10.7$, $+13.6$, and -8.4% under the GFDL, GISS, and UKMO global climate model scenarios, respectively.

10. Interaction Between Temperature and Carbon Dioxide on Growth and Yield of Rice Crop

The atmospheric [CO_2] has been increasing exponentially since the Industrial Revolution. While the atmospheric [CO_2] is increasing by 1.5 μmol mol^{-1} year^{-1}, the global air temperatures are increasing at 0.02 °C year^{-1}. Climate change due to the changes in [CO_2] and temperature has real potential to impact the world's rice production and economies as both elevated [CO_2] and air temperature have significant effects on rice growth and yield. Increasing [CO_2] may influence productivity positively by increasing the amount of carbon available for photosynthesis and negatively by increasing the air temperature due to the greenhouse effect of [CO_2]. For more than three decades, research on the effects of elevated atmospheric [CO_2] alone or in combination with high (elevated) temperature on rice yield and growth has

being carried out (Yoshida, 1973; Imai *et al.*, 1985; Baker *et al.*, 1992; Ziska *et al.*, 1996; Horie *et al.*, 2000; Kim *et al.*, 2003; Baker, 2004; Yang *et al.*, 2006; Sakai *et al.*, 2006; De Costa *et al.*, 2006; Sasaki *et al.*, 2007). Elevated [CO_2] has invariably been found to increase yield (Table 7), while high air temperatures can reduce grain yield even under [CO_2] enrichment (Baker *et al.*, 1992; Ziska *et al.*, 1996; Matsui *et al.*, 1997a; Horie *et al.*, 2000, Prasad *et al.*, 2006) (Fig. 5 and Table 8). Of the many physiological processes affected by these two environmental factors, increased spikelet sterility is considered the foremost (Satake and Yoshida, 1978; Kim *et al.*, 1996b; Matsui *et al.*, 1997a; Oh-e *et al.*, 2007; Jagadish *et al.*, 2007).

The amount and activity of rubisco are often decreased under the elevated atmospheric [CO_2] (Brandner and Salvucci, 2000; Vu *et al.*, 1997). Consequently, there is a suppression of photorespiratory loss of carbon, enhancing net photosynthesis (Brandner and Salvucci, 2000). Hence, there will be more tillers and larger leaves under elevated [CO_2] conditions (Yoshida, 1981; De Costa *et al.*, 2006). Sheehy *et al.* (2001) observed an increasing trend between leaf area and the number of juvenile spikelets. Probably, this is one of the mechanisms whereby elevated [CO_2] could increase yield potential. On the contrary, the temperature of the canopy, which is increased slightly under the elevated [CO_2] can decrease yield (Peng *et al.*, 2004). In the tolerant cultivars, the rate of stomatal conductance of plants, a trait for which genetic variability exists, can modulate the temperature of canopy sufficiently, without adversely affecting the final number of fully formed, mature grains (Matsui *et al.*, 1997b).

The photosynthetic response of rice to different temperature regimes shows considerable variation. There is even a stimulation of single-leaf photosynthesis of rice under high temperature, when plants are subjected to long-term CO_2 treatments during the vegetative stages (Nakagawa *et al.*, 1997). On the contrary, the canopy photosynthesis of rice is found to be relatively unaffected by a range of air temperatures (Baker and Allen, 1993a). Increases in [CO_2] are considered to stimulate rubisco, and with reduction in photorespiration, carbon loss is inhibited. In a single leaf, increasing temperature will support higher net photosynthesis and CO_2 uptake. Further research is warranted on the interaction of [CO_2] and temperature at both vegetative and reproductive stages, paving ways for harnessing the benefits of increasing [CO_2] for higher yields.

11. Screening for High-Temperature Stress Tolerance

From an evolutionary viewpoint, there is availability of variation present in the germplasm, and the variation is also controlled by a significant genetic component. Cultivar differences exist for high-temperature injuries

Table 7 Effect on important physiological parameters and/or their association with high [CO_2] in rice

Physiological parameter	CO_2 cooncentration (μmol mol^{-1})	Impact	Association	Reference
Dark canopy repiration rates	160, 250, 330, 500, 660, and 990	Positive	30–40%	Baker and Allen (1993a)
Days to 50% flowering	330 and 660	Positive	5–20%	Manalo *et al.* (1994)
Days to 50% flowering	350 and 690	Negative	−11%	Kim *et al.* (1996a)
Days to panicle emergence	330 and 660	Positive	1–13%	Manalo *et al.* (1994)
Development rate	160, 250, 330, 500, 660, and 990	Positive	–	Baker and Allen (1993a)
Dry matter production	350 and 690	Positive	15–20%	Kim *et al.* (1996a)
Dry matter production	0.03%, 0.1%, and 0.25%[a]	Positive	20%	Akita and Tanaka (1973)
Dry matter production	175, 350, 1000, and 3500	Positive	20–40%	Imai and Murata (1976)
Dry matter production	330 and 660	Positive	25–33%	Baker *et al.* (1992)
Dry matter production	330 and 660	Positive	8–36%	Baker and Allen (1993b)
Evapotranspiration	160, 250, 330, 500, 660, and 990	Negative	−25%	Baker and Allen (1993a)
Filled grain no. panicle^{-1}	160, 250, 330, 500, 660, and 990	No effect	–	Baker and Allen (1993b)
Filllled spikelets (%)	Ambient, ambient +200, and +300	Negative	−15%	Lin *et al.* (1997)
Filllled spikelets (%)	330 and 660	No effect	–	Baker and Allen (1993b)
Filllled spikelets (%)	300, 1200, and 2400	Positive	9–10%	Yoshida (1976)
Filllled spikelets (%)	350 and 690	Positive	2–4%	Kim *et al.* (1996a)
Flowering duration	330 and 660	Positive	1–2 times	Manalo *et al.* (1994)
Grain mass	160, 250, 330, 500, 660, and 990	No effect	–	Baker and Allen (1993b)
Grain weight	350 and 690	Positive	6%	Kim *et al.* (1996a)
Grain weight	300, 1200, and 2400	Negative	−6%	Yoshida (1976)
Grain no. m^{-2}	300, 1200, and 2400	Positive	100%	Yoshida (1976)
Grain no. panicle^{-1}	300, 1200, and 2400	No effect	–	Yoshida (1976)

(*Continued*)

Table 7 (*Continued*)

Physiological parameter	CO_2 cooncentration (μmol mol^{-1})	Impact	Association	Reference
Harvest index	330 and 660	Positive	21%	Baker *et al.* (1992)
Harvest index	160, 250, 330, 500, 660, and 990	Negative	−50%	Baker and Allen (1993a,b)
Harvest index	350 and 690	Negative	0–6%	Kim *et al.* (1996a)
Leaf area	Ambient, ambient +200, and +300	No effect	–	Lin *et al.* (1997)
Leaf area index	350 and 690	No effect	–	Kim *et al.* (1996a)
Leaf biomass	330 and 660	No effect	–	Manalo *et al.* (1994)
Net canopy photosynthesis	330 and 660	Positive	20%	Rowland-Bamford *et al.* (1996)
Net photosynthesis	330 and 660	Positive	31%	Baker and Allen (1993b)
Nitrogen concentration	160, 250, 330, 500, 660, and 990	Negative	−30 to −40%	Baker and Allen (1993b)
Main-stem leaves (no.)	160, 250, 330, 500, 660, and 990	No effect	–	Baker and Allen (1993a,b)
Panicles (no. m^{-2})	350 and 690	Negative	−13 −21%	Kim *et al.* (1996a)
Panicle number	300, 1200, and 2400	Positive	93	Yoshida (1976)
Panicles (no. $plant^{-1}$)	160, 250, 330, 500, 660, and 990	Positive	12%	Baker and Allen (1993b)
Panicle biomass	Ambient, ambient +200, and +300	Positive	50%	Lin *et al.* (1997)
Panicle biomass	160, 250, 330, 500, 660, and 990	Positive	17%	Baker and Allen (1993a,b)
Phyllochron interval per leaf	330 and 660	Negative	−17%	Manalo *et al.* (1994)
Plant height	350 and 700	No effect	–	Kim *et al.* (1996b)
Plant height	330 and 660	Positive	7–17%	Manalo *et al.* (1994)
Plant height	175, 350, 1000, and 3500	Positive	−8–11%	Imai and Murata (1976)
Plant tissue nitrogen content	160, 250, 330, 500, 660, and 990	Negative	−38–43%	Baker and Allen (1993a)

Photosynthetic rate (Pn)	0.03%, 0.1%, and 0.25%[a]	Positive	45%	Akita and Tanaka (1973)
Photosynthetic rate (Pn)	330 and 600	Positive	40–50%	Vu *et al.* (1997)
Pn with long-term CO_2	160, 250, 330, 500, 660, and 990	Positive	20–30%	Baker and Allen (1993a,b)
Protein content in leaves	350 and 700	Negative	−5–12%	Gesch *et al.* (2003)
Root weight/total weight	350 and 690	Positive	30–40%	Kim *et al.* (1996a)
Root biomass	160, 250, 330, 500, 660, and 990	Positive	30–70%	Baker and Allen (1993b)
Root biomass (g m^{-2})	350 and 690	Positive	70–80%	Kim *et al.* (1996a)
Rubisco activity in leaves	350 and 700	Negative	−14–18%	Gesch *et al.* (2003)
Rubisco activity in leaves	330 and 600	Negative	−12%	Vu *et al.* (1997)
RUBP content, activity	160, 250, 330, 500, 660, and 990	Negative	–	Baker and Allen (1993b)
Specific maintenance respiration	160, 250, 330, 500, 660, and 990	Positive	30–40%	Baker and Allen (1993a)
Specific respiration rate	160, 250, 330, 500, 660, and 990	Negative	−50 −27%	Baker and Allen (1993b)
Spikelets (no. $panicle^{-1}$)	350 and 690	Positive	3–22%	Kim *et al.* (1996a)
Spikelets (no. m^{-2})	350 and 690	Positive	7–10%	Kim *et al.* (1996a)
Stem biomass	330 and 660	Positive	39%	Manalo *et al.* (1994)
Sucrose accumulation rate in leaf	330 and 660	Positive	17%	Rowland-Bamford *et al.* (1996)
Tillers	330 and 660	Positive	25–30%	Baker *et al.* (1992)
Tillers	330 and 660	Positive	14%	Manalo *et al.* (1994)
Tillers	350 and 690	Positive	14–40%	Kim *et al.* (1996a)
Transpiration rate per leaf area	175, 350, 1000, and 3500	Negative	−40 −50%	Imai and Murata (1976)
Total and productive tillers	350 and 690	Positive	15–50%	Kim *et al.* (1996a)
Total and productive tillers	330 and 660	Positive	–	Baker *et al.* (1990)
Total and productive tillers	330 and 660	Positive	14–84%	Manalo *et al.* (1994)
Total biomass	330 and 660	No effect	–	Manalo *et al.* (1994)
Total biomass (g m^{-2})	350 and 690	Positive	70–80%	Kim *et al.* (1996a)
Total duration	160, 250, 330, 500, 660, and 990	Negative	10–12 days	Baker and Allen (1993b)

(*Continued*)

Table 7 (*Continued*)

Physiological parameter	CO_2 cooncentration (μmol mol^{-1})	Impact	Association	Reference
Water loss	160, 250, 330, 500, 660, and 990	Negative	27%	Baker and Allen (1993b)
Water-use efficiency	160, 250, 330, 500, 660, and 990	Positive	52%	Baker and Allen (1993a,b)
Yield	330 and 660	Positive	59%	Baker *et al.* (1992)
Yield	160, 250, 330, 500, 660, and 990	Positive	6%	Baker and Allen (1993b)
Yield	300, 1200, and 2400	Positive	99%	Yoshida (1976)
Yield (g m^{-2})	350 and 690	Positive	20–45%	Kim *et al.* (1996a)

[a] The CO_2 concentration is presented in percentage.

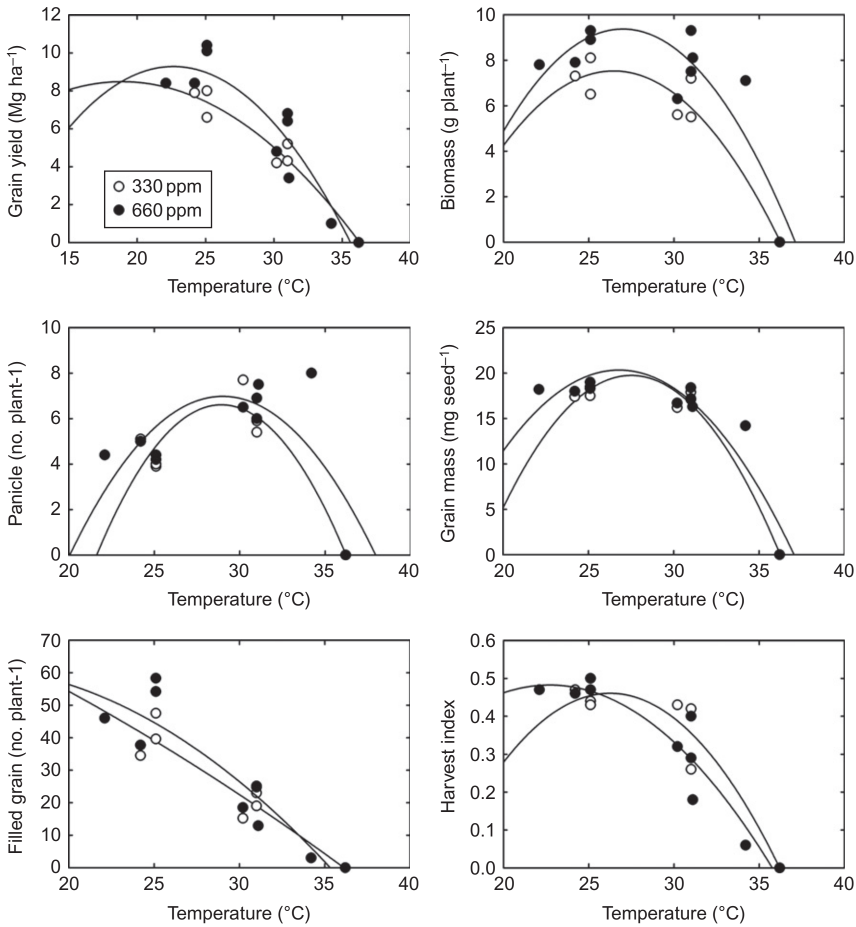


Figure 5 **Temperature and atmospheric CO_2 interactions on rice yield and its components (Baker and Allen, 1993a).**

at different growth stages. Although the genus *Oryza* has a pan-tropical distribution, the geographic origin of rice cultivars is not related to susceptibility to heat stress. For example, BKN6624-46-2, a selection from Thailand, is more susceptible to high temperatures at the vegetative and anthesis stages than the Japanese cv. Fujisaka 5. Different studies conducted by various researchers clearly show the presence of genetic variability among rice cultivars for tolerance to high-temperature stress (Table 9), which needs to be used in the breeding programs.

Many important questions relating to selection of germplasm and exploitation of biodiversity to maximize crop yield remain unanswered

Table 8 Influence of temperature and atmospheric CO_2 concentration on rice crop growth, development, and yield and yield components

Parameter	CO_2 concentration(330 μmol mol^{-1})		CO_2 concentration (660 μmol mol^{-1})	
	Temperature optimum (°C)	Maximum value at the optimum temperature	Temperature optimum (°C)	Maximum value at the optimum temperature
Biomass (g $plant^{-1}$)	26.5	7.86	27.0	9.99
Panicles (no. $plant^{-1}$)	28.6	6.66	29.0	7.45
Grain yield (Mg ha^{-1})	20.0	8.47	22.5	9.43
Harvest index	26.5	0.46	22.5	0.48
Grain mass (mg $seed^{-1}$)	27.5	19.54	26.5	20.85

The optimum temperature and maximum parameter values were estimated from the quadratic fit to the data presented in Fig. 5 (adapted from Baker and Allen, 1993a).

Table 9 Rice genotypic differences in high-temperature tolerance

Stage	Tolerant	Moderately tolerant	Moderately susceptible	Sensitive or susceptible	Reference
Seedling stage	082			Xieqingzao B	Cao and Zhao (2008)
Vegetative				IR26, Calrose, BKN6624-46-2, Pelita I/1	Yoshida *et al.* (1981)
Vegetative	N22			IR8	Roy and Ghosh (1996), Bose and Ghosh (1995), Bose *et al.* (1999)
Vegetative		IR72	M-103		Gesch *et al.* (2003)
Anthesis				C4-63G, Calrose, Pelita i/1, Basmati-370, BKN-6624-46-2	Yoshida *et al.* (1981)
Reproductive				IR24, Calrose	Yoshida *et al.* (1981)
Ripening	Agbede, Carreon, Dular, N22, OS4, PI 215936, Sintiane, Diofor			Basmati-370, BKN 6624-46-2, C4-63G, H4, Pelita 1/1, IR5, IR8, IR20, IR22,	Yoshida *et al.* (1981)

(*Continued*)

Table 9 (*Continued*)

Stage	Tolerant	Moderately tolerant	Moderately susceptible	Sensitive or susceptible	Reference
				IR24, IR26, IR28, IR29, IR30, IR32, IR34, IR36, IR38, IR40, IR42, IR43, IR44, IR45, IR46, IR48, IR50	
Ripening				Tadukan, Tepa-I, Ubaisen, Fujisaka-5	Nagato *et al.* (1966)
Ripening				TN1, IR24, IR26, H4, Fujisaka 5, C4-63g, Pelita I/1	Yoshida *et al.* (1981)
Ripening	N22, IR2006, IET4658			IR1561, IR28, IR52	Mackill *et al.* (1982)
Ripening	Nipponbare			Hinohikari	Matsui *et al.* (2000)
Ripening	Nipponbare, Akitakomachi	Aichinokaori, Yumehikari, Kinmaze, Akhihikari, Aoinokaze		Minamihikari, Hinohikari	Matsui and Omasa (2002) Matsui *et al.* (2001)

Ripening	KRN, Citanduy, Belle patna, BPB			Koshihikari, Sablicun, Tainung 67, Yamada-nishikii	Zakaria *et al.* (2002)
Ripening			Cocodrie, Cypress, Jefferson		Baker (2004)
Ripening	N22		M-103, S-102, Koshihikari, IR8, IR72	L-204, M-202, abelle, WAB-12, Italica Livorna, CG-14, CG-17	Prasad *et al.* (2006)
Ripening	Koshiibuki, Tentakaku			Sasanishiki, Hatsuboshi	Yamakawa *et al.* (2007)
Ripening	Xieyou 46, Guodao 6				Fu *et al.* (2008)
Ripening				Hatsuboshi, Hinohikari	Wakamatsu *et al.* (2008)
Ripening	N22, Bala, IR64, Te qing	CG 14, Co 39, CT9993, IR36, IR62266-24-6-2, Kalinga III, Lemont, Sathi 34-36	Vandana, WAB 56–104, WAB 450-I-B-P38-HB, WAB 450-I-B-P91-HB	Azucena, Moroberekan	Jagadish *et al.* (2008)
Ripening	Shanyou63			Teyou559	Tang *et al.* (2008)
Ripening	Nikomaru Chikushi 64			Hinohikari	Tanaka *et al.* (2009)
Ripening	Huanghuazhan, T226			Shuanggui 1, T219	Cao *et al.* (2009)

(*Continued*)

Table 9 (*Continued*)

Stage	Tolerant	Moderately tolerant	Moderately susceptible	Sensitive or susceptible	Reference
Ripening	N22	IR64		Moroberekan	Jagadish *et al.* (2010)
Grain quality	Fusaotome	Tentakaku, Hanahikari, Koshijiwase	Ajikodama, Kagahikari , Ougiwase, Hitomebore, Haenuki, Hounenwase	Todorokiwase Koshinohana	Ishizaki (2006)
Grain quality				Hinohikari, Koganebare, Hatsuboshi, Mineasahi, Kiho	Wakamatsu *et al.* (2007)

for the predicted futures of elevated/high-temperature conditions (Singh *et al.*, 2007). The phenotypic characters and genetic information to identify useful germplasm, which is crossed to create populations that are then grown and scored for important traits, need prioritization. Unfortunately, identification or selection of material that responds well to elevated temperature and growing the selected material at current temperature is an inadequate approach. There is a definite requirement of experimental environmental facilities. At present, efforts are sporadic on the genetic improvement of rice for high-temperature stress, and the lack of full understanding of how rice plants cope up with high-temperature stress is the main reason for such weak efforts (Singla *et al.*, 1997).

The conservation of rice germplasm and its use in many breeding programs provide many opportunities for evaluation of different germplasm for resistance to high-temperature stress. Wild species, obsolete cultivars, minor varieties, or specialty types of rice are the promising sources harboring genes controlling high-temperature stress tolerance. Additionally, other sources of genes, which include microorganisms, can also be exploited. The innovative biotechnological tools and approaches will help to harness the variations efficiently and to incorporate traits for temperature stress tolerance. Genotypes for flowering and grain filling which are sensitive to high-temperature stress and directly related to yield have been identified (Oh-e *et al.*, 2007; Prasad *et al.*, 2006). Nevertheless, the adverse effects of high temperature are not limited to flowering and fruit set; subsequent development and grain filling are equally affected, resulting in yield reductions.

The systematic evaluation for high-temperature stress tolerance is a costly and time-consuming process. It requires well-defined screening and selection procedures (Singh *et al.*, 2007). Several putative traits might affect the response of rice plants to high-temperature stress. In the target environment, only a few traits contribute to yield. Hence, selection of physiological traits is of paramount importance. Only those traits of known value when combined with selection for yield *per se* can help to achieve the breeding objectives, either in parental selection or in the screening of segregating material.

11.1. Genetic improvement for heat stress tolerance

Rice plants are constantly exposed to a variety of abiotic and biotic stresses. To survive these challenges, they have developed mechanisms to perceive external signals and to manifest adaptive and tolerant responses with proper physiological and morphological changes. Progress in genetic improvements by conventional and molecular breeding approaches has been slow due to the complex physiological responses to heat stress, various other environmental factors, and their interactions. Heat stress tolerance can be defined based on the relative yield of a genotype, compared with other

genotypes subjected to the same stress, and where avoidance is not a major factor. The genotypic comparisons for heat stress tolerance are useful in the context of breeding, using either conventional or molecular approaches, in which both survival and productivity are the major objectives.

The most conventional breeding approaches employ traits such as height, maturity, plant type, pest tolerance, and grain quality in the early screening phase, which is often under optimum conditions. Relatively few genotypes are taken to the advanced testing stage and very few entries are evaluated under the stress conditions of farmers' fields. There is a strong need for early selection under both optimum- and farmers' field conditions. Thus, the testing environments should include the target environment wherein the cultivar will be grown under stress. Complementing the breeding approaches are agronomic practices for greater tolerance to heat stress in important rice-growing regions.

Rice has the smallest genome among the cultivated cereals, and it conserves much of the gene content, and gene order present in other species, to some extent. The full rice genome has now been sequenced (Chen *et al.*, 2002), allowing the identification and localization of genes related to stress tolerance. The rice system can be used to assign function to genes so that homologs can be identified in other species. The systemic relationship between genomes will help the application of functional genomic approaches to rice, in order to understand general plant processes, especially the responses to high-temperature stress.

11.2. Conventional breeding strategies

Conventional breeding methods have depended mainly on the performance of rice such as yield or secondary traits highly associated with yield under stress conditions as a selection criterion. This approach can help to identify cultivars with improved adaptation and performance under stress, but advancement has been slow on genotype × environmental interactions because of year-to-year variations in the timing and intensity of high-temperature stress in fields. Attempts to develop heat-tolerant genotypes via conventional plant breeding protocols are successful and both avoidance and tolerance to heat stresses at anthesis are useful traits for breeding programs for hotter rice-growing environments, now as well as in the future (IRRI, 2007). In many traditional, tropical rice-growing environments, high-temperature tolerance is not an important problem but will become an important breeding objective as there is intensification in dry seasons with irrigation facilities and expansion of rice cultivation in semiarid areas. In the breeding programs, incorporation of high pollen shedding trait into genotypes that are otherwise adapted can be the objective while genotypic differences in pollen germination and pollen tube elongation under high

temperature can be made use. In most cases, high-temperature tolerance at the grain-filling stage is also required.

Extensive information on the response of different cultivars to high temperature under various field conditions as well as their morphological and structural traits can help to select the appropriate best breeding strategies. In Japan, high temperature is causing decreases in grain weight and quality such as transparency, roundness, and cracking in the recent times. Opaque grains caused by high temperatures at the ripening stage are a major constraint for the commercial production of rice. Ishizaki (2006) proposed certain *japonica* cultivars as the standard: Fusaotome for tolerant; Tentakaku, Hanahikari, and Koshijiwase for moderately tolerant; Hitomebore, Haenuki, and Hounenwase for intermediate; Ajikodama, Kagahikari, and Ougiwase for moderately sensitive; and Todorokiwase and Koshinohana for sensitive cultivars. Among the present-day cultivars with heat tolerance at anthesis available, cv. N22 has very high general combining ability (GCA), but its undesirable agronomic traits limit its value as a donor in breeding programs (Mackill *et al*., 1982). Such selection and identification will help to identify the desired traits for heat tolerance.

Besides making selection of cultivars based on maximization of growth and reproductive yield, temperature-resistant flowering, and efficient starch mobilization to the grain, there is also a strong need to ascertain the relationship between [CO_2] and temperature effects on rice growth and development with those on rice productivity (Manalo *et al*., 1994). Prasad *et al*. (2006) suggested that spikelet fertility at high temperature can be used as a screening tool for heat tolerance during reproductive phase. Cao *et al*. (2009) have proposed that pollen fertility acts as an index for heat-tolerance breeding and selecting in rice. As early as 1982, Mackill and others performed diallel cross of rice lines to determine the general and specific combining abilities for the heat-tolerant index, which was calculated by dividing the percentage of filled grains of the heat-treated plants by that of the control plants. Limited attempts have been made so far to develop tolerant cultivars to high-temperature stress. Intensive studies on the morphological and structural traits in cultivars with differential sensitivity to high temperature may provide better understanding of heat tolerance in rice. Improvements of tolerant cultivars and cultivation methods to combat high-temperature stress injury become a continual need (Morita, 2008). Recently, some heat-tolerant rice hybrid Guodao 6 having stable high grain-setting rate and spikelet fertility under high-temperature stress have been identified in China (Tao *et al*., 2008). Manipulation and recombination of the genome into effective combinations using sexual breeding methods are, however, limited by a lack of understanding of interaction among genes. Many traits of interest in rice breeding are quantitatively inherited. Better understanding on the genetic base of multigenic traits using DNA markers is useful in establishing proper breeding strategy.

11.3. Molecular and biotechnological strategies

Molecular mapping and biotechnological strategies offer opportunities to gather information on major genes and quantitative trait loci underlying heat stress tolerance (Table 10). Even though the most important problem with temperature tolerance in rice is pollen (from production to pollination), there are different studies that report heat stress tolerance associated with many different morphological and physiological traits or responses of leaves, stems, reproductive organs, and roots. These responses may be controlled by multiple genes, and presently, there is a limited understanding of the nature of quantitative trait loci for heat tolerance (Huang *et al.*, 2008).

Table 10 Alteration in molecular characters under high-temperature conditions

Molecular parameter	Impact or association	Reference
Expression of the small subunit gene *rbcS*	Downregulated	Gesch *et al.* (2003)
Expression of the small subunit gene *psbA*	Downregulated	Gesch *et al.* (2003)
Sucrose phosphate synthase gene	Upregulated	Hussain *et al.* (1999)
RuBisCO activase precursor (U13)	Downregulated	Han *et al.* (2009)
Proteins related to lignifications	Upregulated	Han *et al.* (2009)
Active antioxidative pathways	Upregulated	Han *et al.* (2009)
HSP-related protection mechanisms	Upregulated	Han *et al.* (2009)
Starch branching enzyme gene SBEI and III	Downregulated	Wei *et al.* (2009b)
Starch branching enzyme gene SBEIV	Upregulated	Wei *et al.* (2009b)
Granule-bound starch synthase I (GBSSI)	Downregulated	Yamakawa *et al.* (2007)
Branching enzymes, especially BEIIb	Downregulated	Yamakawa *et al.* (2007)
Cytosolic pyruvate orthophosphate dikinase	Downregulated	Yamakawa *et al.* (2007)
Expression of prolamin genes	Diminished	Yamakawa *et al.* (2007)
Starch-consuming a-amylases gene	Upregulated	Yamakawa *et al.* (2007)
Heat shock proteins	Upregulated	Yamakawa *et al.* (2007) Jagadish *et al.* (2010)

Zhu *et al.* (2005) detected three quantitative trait loci (QTLs) conferring heat tolerance during grain filling on chromosomes 1, 4, and 7, with logarithm of the odd scores 8, 16, 11.08, and 12.86. Of these, the QTL located in the C1100–R1783 region on chromosome 4 showed no QTL × environment interactions and epistatic effects. Mapping studies will be useful in identifying genetic regions associated with highly heritable traits, and in some cases, it will be possible to identify the specific gene underlying a QTL. Based on the QTL mapping results, research programs can be tailored using marker-assisted selection to validate the usefulness of molecular breeding approaches. The QTL analysis based on yield under stress in breeding materials can do away with screening of component traits in breeding programs.

Exposure to high temperatures and after perception of signals, plants make many changes at the molecular level, including the expression of genes and accumulation of transcripts, and the synthesis of stress-related proteins as a component of a stress tolerance strategy (Iba, 2002). Under mean daily temperature of 32 °C (high temperature) and 22 °C (normal temperature) controlled in growth chambers, the expression responses of eight SSS isoform genes involving starch synthesis metabolism in rice endosperms were detected by Wei *et al.* (2009b). The comparative analysis for the sensitivity of isoform genes exposed to different temperatures can provide the basis for molecular marker-assisted selection in the breeding of heat-tolerant rice cultivars. Heat and drought stress are not synonymous as plants respond to heat or drought differently (Semenov and Halford, 2009). In a recent report, Ginzberg *et al.* (2009) showed that three transcription factors associated with drought responses were actually downregulated in heat-stressed potato plants. Hence, genuine heat stress tolerance markers have to be identified.

Rice may have different responses to heat stress during its lifespan, and the expression of proteins may be altered. Using comprehensive gene screening by a 22-K DNA microarray and differential hybridization, followed by expression analysis by semiquantitative reverse transcription PCR, Yamakawa *et al.* (2007) showed that several starch synthesis-related genes, such as granule-bound starch synthase I (GBSSI) and branching enzymes, especially BEIIb, and a cytosolic pyruvate orthophosphate dikinase gene were downregulated by high temperature, whereas those for starch-consuming α-amylases and Hsps were upregulated when heat stress occurred during the milky stage. High temperature-ripened grains contained decreased levels of amylose and long chain-enriched amylopectin, which might be attributed to the repressed expression of GBSSI and BEIIb, respectively. Likewise, there was a decreased accumulation of 13-kDa prolamin, which was consistent with the diminished expression of prolamin genes under high temperature. Upon heat shock, bulk of these proteins may be localized in the cytoplasm. A novel full-length cDNA encoding for

glycine-rich (GR)-RNA binding protein (RBP, Osgr-rbp4) is isolated from rice heat shock cDNA library by Sahi *et al.* (2007). Amino acid sequence of the deduced protein reveals existence of RNA recognition motif (RRM) comprising of highly conserved RNA binding RNPI and RNPII domains at the N-terminus. C-terminus of this protein is rich in arginine and glycine residues. Sahi *et al.* (2007) suggested that Osgr-rbp4 probably binds and stabilizes the stress-inducible transcripts under heat stress conditions.

Large number of genes may change expression under heat stress, and genomic approaches that can follow transcriptional changes in thousands of genes at a time hold good promise. To investigate gene regulatory mechanisms in the anther in high-temperature environments, Endo *et al.* (2009) performed the DNA microarray analysis and identified the genes responsive to high temperatures from clustering of microarray data. They found that at least 13 were high-temperature-repressed genes in the anther and these genes were expressed specifically in the immature anther, mainly in the tapetum at the microspore stage and downregulated after 1 day of high temperature. However, not all tapetal genes are inhibited by increased temperatures, and high temperatures may disrupt some of the tapetum functions required for pollen adhesion and germination on the stigma. In the proteomic analyses of leaf tissues of 7-day-old rice seedlings, proteins such as lignification-related proteins were found to be regulated by high temperature, and distinct proteins related to protection were upregulated at different high temperatures (Endo *et al.*, 2009). Sohn and Back (2007) showed that transgenic rice plants in which the content of dienoic fatty acids was increased as a result of cosuppression of fatty acid desaturase were more tolerant to high temperatures than untransformed wild-type plants, as judged by growth rate and chlorophyll content. In the literature, reports are now appearing on the changes in the expression of individual genes when rice is exposed to heat stress. Stress-responsive genes as much may not be good targets for crop improvement but those which can respond to signal immediately and are compatible with yield can be very useful. Altering the expression of the useful genes of different pathways through transformation can affect the response of rice and transformation should also aim at improved grain production under heat stress conditions.

12. Experimental Facilities to Characterize High-Temperature Stress Effects

Human struggle for higher control over the environment has continued ever since cultivating plants began. The predicted climate change will pose many new challenges, which include having environmental chambers, even for experimental studies, with desired conditions.

The key purpose of an apparatus for imposing temperature stress is to have precise and accurate control of temperatures and to avoid any deviation of temperatures beyond the controlled experimental temperature. Many physiological processes are also affected by changes in the vapor pressure deficit, which is generally controlled by the RH. In order to characterize the physiological responses to elevated temperature, it is also important to control humidity. Additionally, air movement, which affects plant growth through interaction with temperature; humidity; and CERs need to be optimized. According to Liu *et al.* (2000), an air velocity of 0.5 m s^{-1} is considered optimum for plants under controlled-environment conditions. In the "closed" environments, most atmospheric and soil variables can be adjusted. The "phytotrons," one such "closed environment" and introduced in the 1950s, are useful for research on the interactions of plant and certain environmental variables.

Most studies before 1980 on the effects of high temperature were performed in leaf curettes (LCs), whole plant growth chambers, and greenhouses. In addition to the precisely controlled closed systems, there are open-field exposure systems such as free air concentration enrichment (FACE). The FACE unit is expensive and less precise than the closed systems. New approaches are necessary to reduce the costs of experimental systems and to improve the design, which can characterize temporal dynamics of high-temperature stress processes. At present, some of the experiments using temperature control facilities follow a holistic approach, having experiments in ecosystems as natural as possible and then observing their responses. The modern experimental climate change research facility should allow studies of the interaction of temperature and other variables such as RH on plants, more so under field-like conditions.

12.1. Controlled temperature technologies

There are many temperature-controlled technologies available for conducting experiments under high-temperature conditions, either for plant components individually or for small populations of plants. The functioning of plants in future warmer climates can be appreciated with these new technologies. Despite many limitations, these subnatural high-temperature stress technologies provide opportunities to collect scientific information and make appropriate decisions for identification and selection of cultivars suitable for the future climatic conditions. Some of notable technologies are described below:

12.1.1. Leaf curettes

LCs are designed exclusively for single leaf gas exchange measurements. To study the effect of elevated temperature levels on the CO_2 exchange processes in individual leaves on a short-term basis, the LCs can be used.

12.1.2. Controlled-environment chambers

The CEC is essentially a single chamber. Under the controlled conditions of light intensity and RH, the response of plants to a selected range of constant temperatures can be studied. Depending on the source of light, there are two types: (i) sunlit CEC and (ii) indoor growth chambers or indoor CEC. The sunlit CECs usually have transparent polyester film walls. There are limitations on the control of different environmental variables in CECs. Tashiro and Wardlaw (1991a,b), Manalo *et al.* (1994), and other have used a computer-controlled sunlit environment chambers, while others (Mackill *et al.*, 1982; Lee *et al.*, 2007; Wei *et al.*, 2009a,b; Zhang *et al.*, 2009) have used closed environment chambers in their studies.

12.1.3. Soil–Plant–Atmosphere-Research chambers

The Soil–Plant–Atmosphere-Research (SPAR) chambers provide accurate as well as flexible control of dry bulb temperature, chamber [CO_2], and humidity of the canopy air (Reddy *et al.*, 2001), and extensive functional relationships between plant processes and abiotic factor effects have been derived for modeling (Reddy *et al.*, 1997). The SPAR chambers are sunlit and also provide opportunities for the control and measurement of soil water and root conditions. The ducts have sensors, air sampling ports, and control devices. The air circulated to the top of the canopy can be set to have the prescribed temperature, [CO_2], and humidity levels such facilities were used to quantify interactive effects of temperature and elevated [CO_2] on rice growth, development and yield (Baker and Allen 1993a,b; Baker *et al.*, 1990, 1992), and cultivar responses to temperature (Baker, 2004; Rowland-Bamford *et al.*, 1996).

12.1.4. Temperature-controlled OTCs

The OTCs are designed with blowers with evaporative coolers and in-line heaters with a feedback control system to maintain ambient or increased air temperatures within the chambers. The temperature control system enables conducting the experiments on the interactive effects of air temperature and [CO_2], but with lesser control on other environmental variables. Norby *et al.* (1997), Matsui *et al.* (1997a,b), and Lin *et al.* (1997) have used OTCs to characterize the effects of high temperature on rice spikelet sterility and photosynthetic acclimatization of single leaves of rice.

12.1.5. Temperature gradient chamber

TGCs are essentially an experimental environmental research facility. Generally, the TGC is constructed over field plots, and air drawn through the chambers is heated either by solar radiation or by supplemental heaters. During the day, a temperature gradient along its longitudinal axis is developed using solar energy. At night, the natural diurnal cycle or high-

temperature cycle by heating can be maintained. The gradient of increasing temperature exposes plants inside the chamber to a range of increased temperatures. With the facility for CO_2 enrichment, the TGC can be used for creating various [CO_2] and temperature regimes over the entire growth period (Horie *et al.*, 1995b). The [CO_2] in the TGC is regulated by the air ventilation rate through the TGC and of the [CO_2] release rate (Okada *et al.*, 1995; Sinclair *et al.*, 1995). The control of vapor pressure deficits is difficult to achieve in the present-day TGC facilities. For studies on rice under different levels of atmospheric [CO_2] and temperature, the TGC has been used by many researchers (Horie *et al.*, 1995a; Prasad *et al.*, 2006).

12.1.6. Free air temperature increase technology

The free air temperature increase (FATI) is a new technology to induce increased canopy temperature artificially in field conditions. Without the use of enclosures, the small ecosystems of limited height can be simulated to warm up under field conditions. Infrared heaters are used in FATI, and all radiation below 800 nm is removed by selective cutoff filters to avoid undesirable photomorphogenetic effects (Fig. 6). The ambient canopy temperature in a reference plot (unheated) with thermocouples can be tracked using an electronic control circuit tracks, and the radiant energy from the lamps can be modulated to produce a desired increment in the canopy temperature of an associated heated plot (continuously day and night). This technology is yet to be used in rice, experimental warming of low-stature vegetation can be achieved in a controlled way by irradiation with infrared (0.8–3 μm) both day and night in FATI. Each unit of FATI

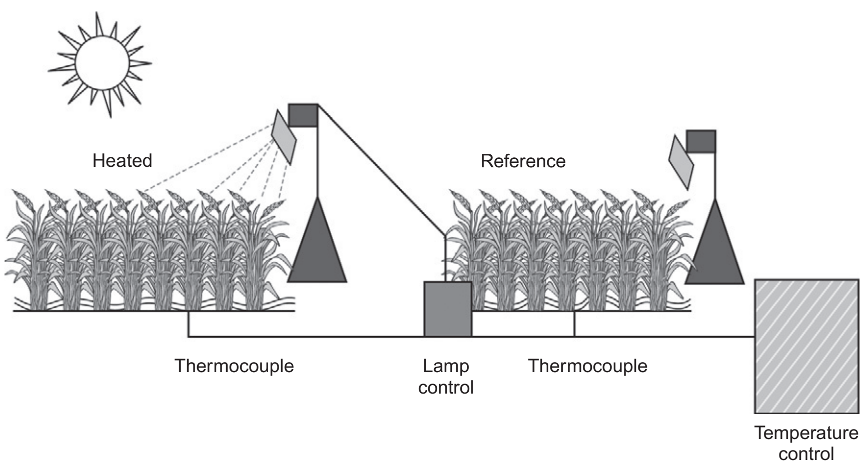


Figure 6 Schematic drawing of the free-air-temperature-increase (FATI) technology used in studying the effects of elevated atmospheric CO_2 and temperature in the field.

consists of a heater, a "dummy" heater without lamps, and an electronic controller that modulates the output of the lamps to maintain constant TD between heated and reference plot (Nijs *et al.*, 1997). In the reference plot, the noncontact semiconductor temperature sensors monitor the temperature. Even if the vegetation is heterogeneous, the temperature increase is highly repeatable in different plots. Without altering microclimate, FATI can be used to study the effects high temperature and other environmental conditions on growth and yield of plants. However, improvements are needed to uniformly warm all plant organs across several layers of the canopy.

13. Mitigation and Adaptation to High-Temperature Stress

Food security is difficult to achieve due to the constant, multifarious struggle by the ever-increasing human population, higher demand and intensification of resource use, and increased per capita consumption (Rosenzweig and Parry, 1994), especially in many Asian countries. With new threats from climate change, there are dilemmas whether rice cultivation needs mitigation options immediately or adaptive measures at high costs or can continue with the "business-as-usual" principle. Increased scientific knowledge on the climate change effects on rice and its cultivation will help to reduce many uncertainties.

13.1. Mitigation

IPCC (2007) defines mitigation as the technological change or substitution that reduces resource inputs and emissions per unit of output. Concerns are more placed on the emission of greenhouse gases. Rice cultivation will not only suffer from the adverse effects of climate change but also contributes to climate change. The submerged rice fields are an important source of greenhouse gas methane. The mitigation technologies should aim at reducing the emission of methane and other greenhouse gases. High temperatures due to climate change are resultant events due to many interlinked activities. Hence, the options for mitigation can encompass many activities which are aimed at reducing the resource inputs and emissions per unit of output. Some of the suggested mitigation options related to rice cultivation are presented below:

- Improved crop and land management to increase soil carbon storage.
- Improved rice cultivation techniques to reduce CH_4 emissions.
- Improved nitrogen fertilizer application techniques to reduce N_2O emissions from rice fields.
- Use of rice straw for replacing fossil fuel use and generation of energy.

- Restoration of degraded lands for rice cultivation.
- Improved energy efficiency.

The mitigation potential of rice production to climate change lies more on its capabilities for soil carbon sequestration; there exists strong synergies with sustainable agriculture. This can reduce vulnerability to high-temperature stress effects in the long run. Another vital aspect of mitigation potential is related to the reductions of CH_4 emission as nearly 50% of rice is cultivated under submerged conditions and human control over the rates of emission from rice fields can be manipulated effectively with different cultivation methods and the use of inputs. Tropical regions provide opportunities for about 65% of the total mitigation potential for climate change, which includes higher temperature stress because more reductions in emission of greenhouse gas methane from rice fields can be achieved.

13.2. Adaptation

According to Matthews and Wassmann (2003), adaptation is an adjustment made within the crop production systems, in order to live successfully with changing climate. The technological changes for adaptation with special reference to rice cultivation will basically aim at the introduction of tolerant cultivars and methods of cultivation for improved input efficiency. The probable adaptive responses need not be new and can include many changes in the current cultivation practices and the use of inputs. With special reference to rice cultivation, the adaptive responses can include the change of planting dates, selection of tolerant cultivars, early maturing cultivars, or high responsive cultivars to inputs, and cultural practices with improved input- and energy efficiencies. The resilience of the production systems needs to be enhanced by these adaptive responses, and salient measures are listed below:

- *Developing tolerant rice cultivars for high-temperature stress*: Breeding cultivars that are tolerant to high-temperature stress should receive utmost importance. Recently, Tao *et al.* (2008) identified rice hybrid Guodao 6 as heat tolerant. Inclusion of tolerant cultivars in the cropping system will be advantageous.
- *Adopting a late or early maturing cultivar and shifting the crop season*: This adaptive measure will benefit immediately under unfavorable conditions of high-temperature stress (Oh-e *et al.*, 2007).
- *Changing planting dates*: Adjustment in sowing dates is a simple yet a powerful tool for adapting to the effects of potential warming (Attri and Rathore, 2003; Baker and Allen, 1993a). Krishnan *et al.* (2007) demonstrated the potential outcomes by adjusting the sowing time of rice in two sites (Cuttack and Jorhat in India) by simulating the crop growth under different climate change scenarios.

- *Pretreatment of rice seedlings*: Pretreating rice seedlings with low levels (<10 μM) of H_2O_2 or NO permits the survival of more green leaf tissue and of higher quantum yield for PSII under heat stress than in nontreated control seedlings (Uchida *et al.*, 2002).
- *Application of chemical substances*: Exogenous applications of osmoprotectants or plant growth-regulating compounds on seeds or whole plants may help the plants to withstand the heat stress. In addition to chemical regulators, fertilizer and irrigation can be used to increase the tolerance to heat stress. The application of chemical substances such as abscisic acid, salicylic acid, and jasmonic acid is found to enhance the tolerance to heat stress in crops. Cao and Zho (2008) used the brassinolide, a new type of plant growth regulator, for treating rice seedlings from heat stress by enhancing the activities or expression level of protective enzymes in leaves.
- *Developing high-temperature-tolerant transgenic rice*: Recombinant DNA technologies offer opportunities for developing high-temperature-tolerant transgenic rice (Katiyar-Agarwal *et al.*, 2003). The rice plants for high-temperature tolerance can be genetically engineered by altering levels of Hsps either directly or through regulatory circuits that govern Hsp levels (Katiyar-Agarwal *et al.*, 2003).
- *Conserving soil moisture*: Additions of crop residues and manure to arable soils will improve the soil water holding capacities.
- *Modification of microclimate*: By providing shelter and shade as in agroforestry systems (Cannell *et al.*, 1996), the effects of extremely high temperatures may be reduced.
- *Establishment of soil covers*: Crop residues or other insulating materials can alter heat transfer for soils. Insulation should not increase the risk of extremely high temperatures for the crops.
- Increasing diversity of crop rotations by choices of species or varieties.
- *Land-use change*: Extensification of existing agricultural land or cultivation of new land or abandonment can improve the resilience of production systems to temperature extremes.

There exists certain benefit from the mitigation or adaptation options to climate change in rice cultivation. Research is warranted to quantify the short- and long-term benefits, suitability, and economic viability of these options to high-temperature stress effects on rice.

14. Conclusion and Future Studies

Rice is food for more than 3 billion people as of today and constitutes the mainstay of food security in many Asian countries. Despite the significance rice has attained, there are continuing struggles with its cultivation for

higher productivity in many countries. The present-day challenges will be exacerbated by changes projected in climate on future rice production. Due to global warming, high-temperature stress will become one of the greatest challenges to rice production. There is an urgent need to understand the growth and development of rice under high-temperature stress, for the purpose of identifying, selecting, and breeding suitable cultivars, which are the utmost important tools to mitigate the adverse effects of high temperature. What is now required is also a portfolio of strategies that includes research, adaptation, mitigation, and technological development in cultivation methods. The developing- and rice-growing countries need to take a proactive role in planning national and regional programs on adaptation and mitigation to climate variability and the predicted climate change as rice will be affected severely in the future climate scenarios, especially in the developing countries.

Tolerant rice cultivars for high-temperature stress will help to avert yield losses to some extent (Krishnan *et al*., 2007). The major food shortages in the future can better be avoided by identifying, selecting, and breeding of suitable rice cultivars. Scientific evidence needs to be collected for the trait identification and breeding methods for high-temperature stress. Heat and drought stresses are often mistakenly assumed to be synonymous but they are not. The responses or genetic control due to heat or drought stress can differ in crops. In heat-stressed potato crop, Ginzberg *et al*. (2009) found that three transcription factors which were associated with drought responses are actually downregulated. There is a strong need to identify the genuine heat stress tolerance markers. Additionally, the scientific information on the effects of high-temperature stress should not be limited to the aboveground portion of rice plants alone. Since the substantial portion of rice plant is in floodwater during the growing season and roots in soils, the effects of high temperatures on floodwater- and soil dynamics, and the ensuing effects on growth and development of rice and nutrient cycling require better understanding. The molecular knowledge of response and tolerance mechanisms is called for developing tools and strategies for engineering rice plants that can tolerate high-temperature stress. Successful cultivation of rice also requires the combination of many other technology options with advanced GIS, climatology tools and decision support systems. Challenges are to develop and disseminate scientific/technical agricultural innovations while time is very short, and there are also substantial uncertainties in the knowledge about the magnitude and occurrence of high-temperature stress. According to Semenov and Halford (2009), there were periods of destructively high temperature in the past, occurring perhaps once every century. Also, there are predictions that such occurrences will be more frequent by the end of this century, occurring perhaps once or twice per decade. What is currently known on the response of rice to high-temperature stress is fragmentary. Future research on how rice plants

respond to high-temperature stress and how tolerance for high temperature can be enhanced is highly warranted.

ACKNOWLEDGMENTS

P. K. thanks J. William Fulbright Foreign Scholarship Board, Washington, USA, for awarding Fulbright Senior Scholar Program Fellowship through United States Educational Foundation in India, New Delhi. Part of this effort (K. R. R.) was funded by the Department of Energy through Sustainable Energy Center, Mississippi State University, Mississippi State, MS, USDA-ARS 58-060402-7-241, and USDA UV-B programs, We thank Drs. Jeff Baker, Jim Bunce, Kenneth Boote, Harry Hodges, and Marybeth Kirkham for their helpful comments and suggestions and Kim Trimm for artwork. This chapter has been approved for publication as Journal Article No. J11853 of the Mississippi Agricultural and Forestry Experiment Station, Mississippi State University.

REFERENCES

Abeysiriwardena, D. S.de.Z., Ohba, K., and Maruyama, A. (2002). Influence of temperature and relative humidity on grain sterility in rice. *J. Natl. Sci. Found. Sri Lanka* **30,** 33–41.

Ahmadi, A., and Baker, D. A. (2001). The effect of water stress on the activities of key regulatory enzymes of the sucrose to starch pathway in wheat. *Plant Growth Regul.* **35,** 81–91.

Ahn, S. B., and Vergara, B. S. (1969). Studies on responses of the rice plant to photoperiod. III. Responses of Korean varieties. *J. Korean Soc. Crop Sci.* **5,** 45–49.

Akita, S. (1989). Improving yield potential in tropical rice. "Progress in Irrigated Rice Research", 971-104-184-7, pp. 41–73. International Rice Research Instittue, Los Baños, Philippines.

Akita, S., and Tanaka, I. (1973). Studies on the mechanism of differences in photosynthesis among species. IV. The differential response in dry matter production between C_3 and C_4 species to atmospheric carbondioxide enrichment. *Proc. Crop Sci. Soc. Jpn.* **42,** 288–295.

Alfonso, M., Yruela, I., Almárcegui, S., Torrado, E., Pérez, M. A., and Picorel, R. (2001). Unusual tolerance to high temperature in a new herbicide-resistant D1 mutant from *Glycine max* (L.) *Merr.* cell cultures deficient in fatty acid desaturation. *Planta* **212,** 573–582.

Al-Khatib, K., and Paulsen, G. M. (1999). High-temperature effects on photosynthetic processes in temperate and tropical cereals. *Crop Sci.* **39,** 119–125.

Amthor, J. S. (1986). Evolution and applicability of a whole plant respiration model. *J. Theor. Biol.* **122,** 473–490.

Andrews, C. H. (1982). Preharvest environment: Weathering. *In* "Proceedings of a Conference for Scientists of Asia" (J. B. Sinclair and J. A. Jackobs, Eds.), pp. 19–25. Soybean Seed Quality and Stand Establishment, Colombo, Sri Lanka.

Asakuma, S., and Iwashita, T. (1961). Ecological studies of heading of rice. III. Some experiments about the restraint of heading by high temperature. *Proc. Crop Sci. Soc. Jpn.* **29,** 334–336.

Attri, S. D., and Rathore, L. S. (2003). Simulation of impact of projected climate change on wheat in India. *Int. J. Clim.* **23,** 693–705.

Azmi, A. R. (1969). Photoperiod and temperature effects on the growth and development of rice (*Oryza sativa* L.*)*. PhD Thesis, University of British Columbia, 182pp.

Baker, J. T. (2004). Yield responses of southern US rice cultivars to CO_2 and temperature. *Agric. For. Meteorol.* **122,** 129–137.

Baker, J. T., and Allen, L. H. Jr. (1993a). Effects of CO_2 and temperature on rice: A summary of five growing seasons. *J. Agri. Meterol.* **48,** 575–582.

Baker, J. T., and Allen, L. H. Jr. (1993b). Contrasting crop species responses to CO_2 and temperature: Rice, soybean and citrus. *Vegetatio* **104**(105), 239–260.

Baker, J. T., Allen, L. H. Jr., and Boote, K. J. (1990). Growth and yield response of rice to carbon dioxide concentration. *J. Agri. Sci. (Camb.)* **115,** 313–320.

Baker, J. T., Allen, L. H. Jr., and Boote, K. J. (1992). Response of rice to carbon dioxide and temperature. *Agri. For. Meteorol.* **60,** 153–166.

Baker, J. T., Kim, S., Gitz, D. C., Timlin, D. J., and Reddy, V. R. (2005). Photosynthesis and yield of southern US rice cultivars in response to CO_2 and temperature. *J. Agri. Meteorol.* **60,** 457–462.

Banon, S., Fernandez, J. A., Franco, J. A., Torrecillas, A., Alarcon, J. J., and Sanchez-Blanco, M. J. (2004). Effects of water stress and night temperature preconditioning on water relations and morphological and anatomical changes of *Lotus creticus* plants. *Sci. Hortic.* **101,** 333–342.

Battisti, D. S., and Naylor, R. L. (2009). Historical warnings of future food insecurity with unprecedented seasonal heat. *Science* **323,** 240–244.

Bose, A., and Ghosh, B. (1995). Effect of heat stress on ribulose 1,5-biphosphate carboxylase in rice. *Phytochemistry* **38,** 1115–1118.

Bose, A., Tiwari, R. S., Chattopadhyay, M. K., Gupta, S., and Ghosh, B. (1999). Thermal stress induces differential degradation of Rubsico in heat sensitive and heat tolerant rice. *Physiol. Plant.* **105,** 89–94.

Bowes, G. (1993). Facing the inevitable: Plants and increasing atmospheric CO_2. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **44,** 309–332.

Brandner, S. J. C., and Salvucci, M. E. (2000). Rubisco activase constrains the photosynthetic potential of leaves at high temperature and CO_2. *Proc. Natl. Acad. Sci. USA* **97,** 13430–13435.

Burroughs, W. (2003). Climate into the 21st Century. Cambridge University Press, Cambridge, 240pp.

Cannell, M. G. R., van Noordwijk, M., and Ong, C. K. (1996). The central agroforestry hypothesis: The trees must acquire resources that the crop would not otherwise acquire. *Agrofor. Syst.* **34,** 27–31.

Cao, Y. Y., and Zhao, H. (2008). Protective roles of brassinolide on rice seedlings under high temperature stress. *Rice Sci.* **15,** 63–68.

Cao, Y. Y., Duan, H., Yang, L. N., Wang, Z. Q., Zhou, S. C., and Yang, J. C. (2008). Effect of heat stress during meiosis on grain yield of rice cultivars differing in heat tolerance and its physiological mechanism. *Acta Agron. Sin.* **34,** 2134–2142.

Cao, Y. Y., Duan, H., Yang, L. N., Wang, Z. Q., Liu, L. J., and Yang, J. C. (2009). Effect of high temperature during heading and early filling on grain yield and physiological characteristics in *Indica* rice. *Acta Agron. Sin.* **35,** 512–521.

Challinor, A. J., Wheeler, T. R., Craufurd, P. Q., and Slingo, J. M. (2005). Simulation of the impact of high temperature stress on annual crop yields. *Agric. For. Meteorol.* **135,** 180–189.

Chaudhary, T. N., and Ghildyal, B. P. (1970). Influence of submerged soil temperature regimes on growth, yield, and nutrient composition of rice plant. *Agron. J.* **62,** 281–285.

Chen, M., Presting, G., Barbazuk, W. B., Goicoechea, J. L., Blackmon, B., Fang, G., Kim, H., Frisch, D., Yu, Y., Sun, S., Higinbottom, S., Phimphilai, J., *et al.* (2002). An integrated physical and genetic map of the rice genome. *Plant Cell* **14,** 537–545.

Cheng, F., Zhong, L., Zhao, N., Liu, Y., and Zhang, G. (2005). Temperature induced changes in the starch components and biosynthetic enzymes of two rice varieties. *Plant Growth Regul.* **46,** 87–95.

Cheng, W., Sakai, H., Yagi, K., and Hasegawa, T. (2009). Interactions of elevated [CO_2] and night temperature on rice growth and yield. *Agric. For. Meteorol.* **149,** 51–58.

Childs, N., and Baldwin, K. (2010). Rice outlook. A report from the Economic Research Service, http://usda.mannlib.cornell.edu/usda/current/RCS/RCS-01-13-2010.pdf.

Chin, K. J., and Conrad, R. (1995). Intermediary metabolism in methanogenic paddy soil and influence of temperature. *FEMS Microbiol. Ecol.* **18,** 85–102.

Cho, J. (1956). Double fertilization in *Oryza sativa* L. and development of the endosperm with special reference to the aleurone layer. *Bull. Natl. Inst. Agric. Sci.* **6,** 61–101.

Christiansen, M. N. (1978). The physiology of plant tolerance to temperature extremes. *In* "Crop Tolerance to Suboptimal land Conditions" (G. A. Jung, Ed.), pp. 173–191. American Society of Agronomy, Madison, WI.

Coffman, W. R. (1977). Rice varietal development for cropping systems at IRRI [Philippines]. "Proceedings of Symposium on Cropping Systems Research and Development for the Asian Rice Farmer, Philippines", pp. 359–371. International Rice Research Institute, Los Banos, Philippines.

Cohen, J. E. (2003). Human population: The next half century. *Science* **302,** 1172–1175.

Confalonieri, R., Mariani, L., and Bocchi, S. (2005). Analysis and modelling of water and near water temperatures in flooded rice (*Oryza sativa* L.). *Ecol. Model.* **183,** 269–280.

Cooper, N. T. W., Siebenmorgen, T. J., and Counce, P. A. (2008). Effects of nighttime temperature during kernel development on rice physicochemical properties. *Cereal Chem.* **85,** 276–282.

Counce, P. A., Bryant, R. J., Bergman, C. J., Bautista, R. C., Wang, Y.-J., Siebenmorgen, T. J., Moldenhauer, K. A. K., and Meullenet, J. F. C. (2005). Rice milling quality, grain dimensions, and starch branching as affected by high night temperatures. *Cereal Chem.* **82,** 645–648.

Daniels, M. J., Marks, B. P., Siebenmorgen, T. J., McNew, R. W., and Meullenet, J.-F. (1998). Effects of long-grain rough rice storage history on end-use quality. *J. Food Sci.* **63,** 832–835.

Dawe, D. (2007). Agricultural research, poverty alleviation and key trends in Asia's rice economy. *In* "Charting New Pathways to C_4 Rice" (J. E. Sheehy, P. L. Mitchell, and B. Hardy, Eds.), pp. 37–53. International Rice Research Institute, Los Banos, Philippines.

De Costa, W. A. J. M., Weerakoon, W. M. W., Herath, H. M. L. K., Amaratunga, K. S. P., and Abeywardena, R. M. I. (2006). Physiology of yield determination of rice under elevated carbon dioxide at high temperatures in a subhumid tropical climate. *Field Crops Res.* **96,** 336–347.

De Datta, S. K. (1981). Principles and Practices of Rice Production. John Wiley and Sons, New York, 618pp.

Dillahunty, A. L., Siebenmorgen, T. J., Buescher, R. W., Smith, D. E., and Mauromoustakos, A. (2000). Effect of moisture content and temperature on respiration rate of rice. *Cereal Chem.* **77,** 541–543.

Downey, D. A., and Wells, B. R. (1975). Air temperatures in the Starbonnet rice canopy and their relationship to nitrogen timing, grain yield and water temperature. *Univ. Arkansas Agric. Exp. Stn. Bull.* No. 796, 27pp.

Easterling, D. R., Horton, B., Jones, P. D., Peterson, T. C., Karl, T. R., Parker, D. E., Salinger, M. J., Razuvayev, V., Plummer, N., Jamason, P., and Folland, C. K. (1997). Maximum and minimum temperature trends for the globe. *Science* **277,** 364–367.

Easterling, W. E., Aggarwal, P. K., Batima, P., Brander, K. M., Erda, L., Howden, S. M., Kirilenko, A., Morton, J., Soussana, J.-F., Schmidhuber, J., and Tubiello, F. N. (2007). Food, fibre and forest products. *In* "Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change" (M. L. Parry, O. F. Canziani,

J. P. Palutikof, P. J. van der Linden, and C. E. Hanson, Eds.), pp. 273–313. Cambridge University Press, Cambridge, United Kingdom.

Egeh, A. O., Ingram, K. T., and Zamora, O. B. (1992). High temperature effects on leaf gas exchange of four rice cultivars. *Philipp. J. Crop Sci.* **17,** 21–26.

Ekanayake, I. J., Steponkus, P. L., and De Datta, S. K. (1989). Spikelet sterility and flowering response of rice to water stress at anthesis. *Ann. Bot.* **63,** 257–264.

Ellis, R. H., and Hong, T. D. (1994). Desiccation tolerance and potential longevity of developing seeds of rice (*Oryza sativa* L.). *Ann. Bot.* **73,** 501–506.

Ellis, R. H., and Pieta Filho, C. (1992). Potential longevity of seeds in storage, in relation to maturity. *Seed Sci. Res.* **2,** 9–15.

Ellis, R. H., Qi, A., Summerfield, R. J., and Roberts, E. H. (1993). Rates of leaf appearance and panicle development in rice (*Oryza sativa* L.): A comparison at three temperatures. *Agric. For. Meteorol.* **66,** 129–138.

Endo, M., Tsuchiya, T., Hamada, K., Kawamura, S., Yano, K., Ohshima, M., Higashitani, A., Watanabe, M., and Kobayashi, M. K. (2009). High temperatures cause male sterility in rice plants with transcriptional alterations during pollen development. *Plant Cell Physiol.* **50,** 1911–1922.

Enomoto, N., Yamada, I., and Hozumi, K. (1956). On the artificial germination of pollen in rice plants. IV. Temperature limits of pollen germination in rice varieties. *Jpn. J. Crop Sci.* **25,** 69.

FAO. (2008). Food and agriculture Statistics Global Outlook. Food and Agricultural Organization of the United Nations, http://faostat.fao.org/Portals/_Faostat/documents/pdf/world.pdf.

Fitzgerald, M. A., and Resurreccion, A. P. (2009). Maintaining the yield of edible rice in a warming world. *Funct. Plant Biol.* **36,** 1037–1045.

Foolad, M. R. (2005). Breeding for abiotic stress tolerances in tomato. *In* "Abiotic Stresses: Plant Resistance Through Breeding and Molecular Approaches" (M. Ashraf and P. J. C. Harris, Eds.), pp. 613–684. The Haworth Press Inc., New York, USA.

Fourré, J. L., and Lhoest, J. (1989). Protein synthesis and modification by heat in rice cell culture. *Plant Sci.* **61,** 69–74.

Fu, G., Tao, L., Song, J., Wamg, X., Cao, L., and Cheng, S. (2008). Responses of yield characteristics to high temperature during flowering stage in hybrid rice Guodao 6. *Rice Sci.* **15,** 215–222.

Funaba, M., Ishibashi, Y., Molla, A. H., Iwanami, K., and Inoue, M. I. (2006). Influence of low/high temperature on water status in developing and maturing rice grains. *Plant Prod. Sci.* **9,** 347–354.

Gao, L., Jun, Z., Huang, Y., and Zhang, L. (1992). Rice clock model—A computer model to simulate rice development. *Agric. For. Meteorol.* **60,** 1–16.

Gesch, R. W., Vu, J. C. V., Allen, L. H. Jr., and Boote, K. J. (2001). Photosynthetic responses of rice and soybean to elevated CO_2 and temperature. *Recent Res. Dev. Plant Physiol.* **2,** 125–137.

Gesch, R. W., Kang, I.-H., Gallo-Meagher, M., Vu, J. C. V., Boote, K. J., Allen, L. H. Jr., and Bowes, G. (2003). Rubisco expression in rice leaves is related to genotypic variation of photosynthesis under elevated growth CO_2 and temperature. *Plant Cell Environ.* **26,** 1941–1950.

Ginzberg, I., Barel, G., Ophir, R., Tzin, E., Tanami, Z., Muddarangappa, T., de Jong, W., and Fogelman, E. (2009). Transcriptomic profiling of heat-stress response in potato periderm. *J. Exp. Bot.* **60,** 4411–4421.

Grist, D. H. (1986). Rice. Longman Singapore Publishers, Singapore, 599pp.

Guilioni, L., Wery, J., and Tardieu, F. (1997). Heat stress-induced abortion of buds and flowers in pea: Is sensitivity linked to organ age or to relations between reproductive organs? *Ann. Bot.* **80,** 159–168.

Guo, P. G., and Li, R. H. (2000). Effects of high nocturnal temperature on photosynthetic organization in rice leaves. *Acta Bot. Sin.* **42,** 673–678.

Halford, N. G. (2009). New insights on the effects of heat stress on crops. *J. Exp. Bot.* **60,** 4215–4216.

Hall, A. E. (1992). Breeding for heat tolerance. *Plant Breed Rev.* **10,** 129–168.

Halwart, M., and Gupta, M. V. (2004). Culture of fish in rice fields. FAO and The World Fish Center, Italy and Malaysia.

Han, F., Chen, H., Li, X. J., Yang, M. F., Liu, G. S., and Shen, S. H. (2009). A comparative proteomic analysis of rice seedlings under various high-temperature stresses. *Biochim. Biophys. Acta* **1794,** 1625–1634.

Harrington, J. F. (1972). Seed storage and longevity. *In* "Seed Biology" (T. T. Kozlowski, Ed.), pp. 142–245. Academic Press, New York.

Hartmann, H. T., Kester, D. E., Davies, F. T. Jr., and Geneve, R. L. (1997). Plant Propagation Principles and Practices. Prentice Hall, New Jersey, 770pp.

Henderson, L. (1934). Relation between root respiration and absorption. *Plant Physiol.* **9,** 283–300.

Hirano, H. Y., and Sano, Y. (1998). Enhancement of *Wx* gene expression and the accumulation of amylose in response to cool temperatures during seed development in rice. *Plant Cell Physiol.* **39,** 807–812.

Hirose, T., Endler, A., and Ohsugi, R. (1999). Gene expression of enzymes for starch and sucrose metabolism and transport in leaf sheath of rice (*Oryza sativa* L.) during the heading period in relation to the sink to source transition. *Plant Prod. Sci.* **2,** 178–183.

Hori, K., Purboyo, R. B. R. A., Akinaga, Y., Okita, T., and Itoh, K. (1992). Knowledge and preference of aromatic rice by consumers in East and South-east Asia. *J. Consum. Stud. Home Econ.* **16,** 199–206.

Horie, T., Nakagawa, H., Centeno, H. G. S., and Kropff, M. J. (1995a). The rice simulation model SIMRIW and its testing. *In* "Modeling the Impact of Climate Change on Rice Production in Asia" (R. B. Matthews, M. J. Kropff, D. Bachelet, and H. H. van Laar, Eds.), pp. 51–66. CAB International, Wallingford, United Kingdom and International Rice Research Institute, Manila, Philippines.

Horie, T., Nakagawa, H., Nakano, J., Hamotani, K., and Kim, H. Y. (1995b). Temperature gradient chambers for research on global environment change. III. A system designed for rice in Kyoto, Japan. *Plant Cell Environ.* **18,** 1064–1069.

Horie, T., Baker, J. T., Nakagawa, H., Matsui, T., and Kim, H. Y. (2000). Crop ecosystem responses to climatic change: Rice. *In* "Climate Change and Global Crop Productivity" (K. R. Reddy and H. F. Hodges, Eds.), pp. 81–106. CAB International, Wallingford, UK.

Hosoi, N., and Tamagata, N. (1973). The study of interaction of environmental factors for rice plant heading. *Jpn. J. Breed.* **23,** 110–111.

Howarth, C. J. (2005). Genetic improvement of growth and survival at high temperature. *In* "Abiotic Stresses: Plant Resistance Through Breeding and Molecular Approaches" (M. Ashraf and P. J. C. Harris, Eds.), pp. 277–300. The Haworth Press Inc., New York.

Hu, W. H., Hu, G. C., and Han, B. (2009). Genome-wide survey and expression profiling of heat shock proteins and heat shock factors revealed overlapped and stress specific response under abiotic stresses in rice. *Plant Sci.* **176,** 583–590.

Huang, J., Wang, M. M., Jiang, Y., Bao, Y. M., Huang, X., Sun, H., Xu, D. Q., Lan, H. X., and Zhang, H. S. (2008). Expression analysis of rice A20/AN1-type zinc finger genes and characterization of ZFP177 that contributes to temperature stress tolerance. *Gene* **420,** 135–144.

Hussain, M. W., Allen, L. H. Jr., and Bowes, G. (1999). Up-regulation of sucrose phosphate synthase in rice grown under elevated CO_2 and temperature. *Photosynth. Res.* **60,** 199–208.

Iba, K. (2002). Acclimative response to temperature stress in higher plants: Approaches of gene engineering for temperature tolerance. *Annu. Rev. Plant Biol.* **53,** 225–345.

Imai, K., and Murata, Y. (1976). Effect of carbon dioxide concentration on growth and dry matter production in crop plants. I. Effects on leaf area, dry matter, tillering, dry matter distribution ratio, and transpiration. *Jpn. J. Crop Sci.* **45,** 598–606.

Imai, K., Coleman, D. F., and Yanagisawa, T. (1985). Increase in atmospheric partial pressure of carbon dioxide and growth and yield of rice (*Oryza sativa* L.). *Jpn. J. Crop Sci.* **54,** 413–418.

Imaki, T., Jyokei, K., and Yamada, I. (1983). Sterility caused by high temperature at flowering in rice plants. *Bull. Fac. Agric. Shimane Univ.* **17,** 1–7.

Inaba, K. (2005). Ultraviolet-B radiation (UV-B) under high-temperature conditions affects growth of rice (cv.Kosihikari) after a young panicle formation stage. *Jpn. J. Crop Sci.* **74,** 200–206.

Inaba, K., and Sato, K. (1976). High temperature injury of ripening in rice plant. VI. Enzyme activities of kernel as influenced by high temperature. *Proc. Crop Sci. Soc. Jpn.* **45,** 162–167.

IPCC (Intergovernmental Panel on Climate Change (2007). Climate Change 2007: Impacts, adaptation and vulnerability.(M. L. Parry, O. F. Canziani, J. P. Palutikof, P. J. van der Linden, and C. E. Hanson, Eds.), *In* "Contribution of Working Group II to Fourth Assessment Report of the Intergovernmental Panel on Climate Change". Cambridge University Press, Cambridge, United Kingdom, 1000pp.

IRRI (1978). International Rice Research Institute Annual Report for 1977 International Rice Research Institute, Manila, Philippines, P.O. Box 933, 548pp.

IRRI (1979). International Rice Research Institute Philippines, P.O Annual Report for 1978 International Rice Research Institute, Manila, Philippines, P.O. Box 933, 478pp.

IRRI (2007). International Rice Research Institute. Annual Report for 2007 IRRI, P.O. Box 933, Manila, Philippineshttp://beta.irri.org/index.php/Programs/List-of-IRRI-Research-Programs/Annual-Report-2007.html.

Ishimaru, T., Horigane, A. K., Ida, M., Iwasawa, N., San-oh, Y. A., Nakazono, M., Nishizawa, N. K., Masumura, T., Kondo, M., and Yoshida, M. (2009). Formation of grain chalkiness and changes in water distribution in developing rice caryopses grown under high-temperature stress. *J. Cereal Sci.* **50,** 166–174.

Ishizaki, K. (2006). Evaluation of various screening systems for high grain quality in rice cultivars under high-temperature grain-filling conditions, and the selection of their standard cultivars. *Jpn. J. Crop Sci.* **75,** 502–506.

Islam, M. S., and Morison, J. I. L. (1992). Influence of solar radiation and temperature on irrigated rice grain yield in Bangladesh. *Field Crops Res.* **30,** 13–28.

Ito, S., Hara, T., Kawanami, Y., Watanabe, T., Thiraporn, K., Ohtake, N., Sueyoshi, K., Mitsui, T., Fukuyama, T., Takahashi, Y., Sato, T., Sato, A., *et al.* (2009). Carbon and nitrogen transport during grain filling in rice under high-temperature conditions. *J. Agron. Crop Sci.* **195,** 368–376.

Jagadish, S. V. K., Craufurd, P. Q., and Wheeler, T. R. (2007). High temperature stress and spikelet fertility in rice (*Oryza sativa L.*). *J. Exp. Bot.* **58,** 1627–1635.

Jagadish, S. V. K., Craufurd, P. Q., and Wheeler, T. R. (2008). Phenotyping parents of mapping populations of rice for heat tolerance during anthesis. *Crop Sci.* **48,** 1140–1146.

Jagadish, S. V. K., Muthurajan, R., Oane, R., Wheeler, T. R., Heuer, S., Bennett, J., and Craufurd, P. Q. (2010). Physiological and proteomic approaches to address heat tolerance during anthesis in rice (*Oryza sativa* L.). *J. Exp. Bot.* **61,** 143–156.

Jang, E. H., Lim, S. T., and Kim, S. S. (2009). Effect of storage temperature for paddy on consumer perception of cooked rice. *Cereal Chem.* **86,** 549–555.

Jeng, T. L., Tseng, T. H., Wang, C. S., Chen, C. L., and Sung, J. M. (2003). Starch biosynthesizing enzymes in developing grains of rice cultivar Tainung 67 and its sodium azide-induced rice mutant. *Field Crop Res.* **84,** 261–269.

Jiang, H., Dian, W., and Wu, P. (2003). Effect of high temperature on fine structure of amylopectin in rice endosperm by reducing the activity of the starch branching enzyme. *Phytochemistry* **63,** 53–59.

Jin, Z. X., Yang, J., Qian, C. R., Liu, H. Y., Jin, X. Y., and Qiu, T. Q. (2005). Effects of temperature during grain filling period on activities of key enzymes for starch synthesis and rice grain quality. *Chin. J. Rice Sci.* **19,** 377–380.

Jones, P. D., New, M., Parker, D. E., Martin, S., and Rigor, I. G. (1999). Surface air temperature and its changes over the past 150 years. *Rev. Geophys.* **37,** 173–199.

Kameswara Rao, N., and Jackson, M. T. (1996). Variation in seed longevity of rice cultivars belonging to different isozyme groups. *Genet. Res. Crop Evol.* **44,** 159–164.

Katiyar-Agarwal, S., Agarwal, M., and Grover, A. (2003). Heat-tolerant basmati rice engineered by over-expression of hsp101. *Plant Mol. Biol.* **51,** 677–686.

Khakwani, A. A., Shiraishi, M., Zubair, M., Baloch, M. S., Naveed, K., and Awan, I. (2005). Effect of seedling age and water depth on morphological and physiological aspects of transplanted rice under high temperature. *J. Zhejiang Univ. Sci.* **6,** 389–395.

Khush, G. S. (2005). What it will take to feed 5.0 billion rice consumers in 2030. *Plant Mol. Biol.* **59,** 1–6.

Kim, H. Y., Horie, T., Nakagawa, H., and Wada, K. (1996a). Effects of elevated CO_2 concentration and high temperature on growth and yield of rice. I. The effect on development, dry matter production and some growth characteristics. *Jpn. J. Crop Sci.* **65,** 634–643.

Kim, H. Y., Horie, T., Nakagawa, H., and Wada, K. (1996b). Effects of elevated CO_2 concentration and high temperature on growth and yield of rice. II. The effect on yield and its components on Akihikari rice. *Jpn. J. Crop Sci.* **65,** 644–651.

Kim, H. Y., Lieffering, M., Kobayashi, K., Okada, M., Mitchell, M. W., and Gumpertz, M. (2003). Effects of free-air CO_2 enrichment and nitrogen supply on the yield of temperate paddy rice crops. *Field Crops Res.* **83,** 261–270.

Klepper, B., Rickman, R. W., and Peterson, C. M. (1982). Quantitative characterization of vegetative development in small cereal grains. *Agron. J.* **74,** 789–792.

Kobata, T., and Uemuki, N. (2004). High temperatures during the grain-filling period do not reduce the potential grain dry matter increase of rice. *Agron. J.* **96,** 406–414.

Kobata, T., Uemuki, N., Inamura, T., and Kagata, H. (2004). Shortage of assimilate supply to grain increases the proportion of milky white rice kernels under high temperatures. *Jpn. J. Crop Sci.* **73,** 315–322.

Kondo, M., and Okamura, T. (1931). Growth response of rice plant to water temperature. *Agric. Hortic.* **6,** 517–530.

Krishnan, P., and Surya Rao, A. V. (2005). Effects of genotype and environment on seed yield and quality of rice. *J. Agric. Sci. (Camb.)* **143,** 283–292.

Krishnan, P., Swain, D. K., Bhaskar, B. C., Nayak, S. K., and Dash, R. N. (2007). Impact of elevated CO_2 and temperature on rice yield and methods of adaptation as evaluated by crop simulation studies. *Agric. Ecosyst. Environ.* **122,** 233–242.

Krishnan, P., Ramakrishnan, B., Rao, K. S., and Dash, R. N. (2008). Simulation studies to characterize the impact of climate change on crop production and to identify strategies for adaptation and mitigation. *In* “Climate Change and Crops” (S. N. Singh, Ed.), pp. 39–61. Springer Verlag, Germany.

Kubo, A., Fujita, N., Harada, K., Matsuda, T., Satoh, H., and Nakamura, Y. (1999). The starch-debranching enzymes isoamylase and pullulanase are both involved in amylopectin biosynthesis in rice endosperm. *Plant Physiol.* **121,** 399–409.

Kukla, G., and Karl, T. R. (1993). Nighttime warming and the greenhouse effect. *Environ. Sci. Technol.* **27,** 1468–1474.

Kunze, O. R. (1977). Moisture adsorption influences on rice. *J. Food Proc. Eng.* **1,** 167–181.

Lambers, H. (1985). Respiration in intact plants and tissues: Its regulation and dependence on environmental factors, metabolism and invaded organism. *In* "Higher Plant Cell Respiration" (R. Douce and D. A. Day, Eds.), Encyclopedia of Plant Physiology. New Ser., Vol. 18, pp. 418–473. Springer, Berlin.

Lee, D. G., Ahsan, N., Lee, S. H., Kang, K. Y., Bahk, J. D., Lee, I. J., and Lee, B. H. (2007). A proteomic approach in analyzing heat responsive proteins in rice leaves. *Proteomics* **7,** 3369–3383.

Li, X. Z., Liang, M. Z., Zhou, G. Q., and Chen, L. B. (2002). Effect of environment condition on pollen vigor and seed set during flowering time of rice. *Acta Agron Sin.* **28,** 417–420.

Lin, W., Ziska, L. H., Namuco, O. S., and Bai, K. (1997). The interaction of high temperature and elevated CO_2 on photosynthetic acclimation of single leaves of rice *in situ*. *Physiol. Plant.* **99,** 178–184.

Lisle, A. J., Martin, M., and Fitzgerald, M. A. (2000). Chalky and translucent rice grains differ in starch composition and structure and cooking properties. *Cereal Chem.* **77,** 627–632.

Liu, L., Hoogenboom, G., and Ingram, K. T. (2000). Controlled-environment sunlit plant growth chambers. *Crit. Rev. Plant Sci.* **19,** 347–375.

Livingston, B. E., and Haasis, F. W. (1933). Relations of time and maintained temperature to germination percentage for a lot of rice seed. *Am. J. Bot.* **20,** 596–615.

Long, S. P. (1991). Modification of the response of photosynthetic productivity to rising temperature by atmospheric CO_2 concentrations: Has its importance been underestimated? *Plant Cell Environ.* **14,** 729–739.

Mackill, D. J., Coffman, W. R., and Rutger, N. J. (1982). Pollen shedding and combining ability for high temperature tolerance in rice. *Crop Sci.* **22,** 730–733.

Manalo, P. A., Ingram, K. T., Pamplona, R. R., and Egeh, A. O. (1994). Atmospheric carbon dioxide and temperature effects on rice. *Agric. Ecosyst. Environ.* **51,** 339–347.

Marcum, K. B. (1998). Cell membrane thermostability and whole plant heat tolerance of Kentucky bluegrass. *Crop Sci.* **38,** 1214–1218.

Matsui, T. (2009). Floret sterility induced by high temperatures at the flowering stage in rice (*Oryza sativa* L.). *Jpn. J. Crop Sci.* **78,** 303–311.

Matsui, T., and Omasa, K. (2002). Rice (*Oryza sativa* L.) cultivars tolerant to high temperature at flowering: Anther characteristics. *Ann. Bot.* **89,** 683–687.

Matsui, T., Namuco, O. S., Ziska, L. H., and Horie, T. (1997a). Effects of high temperature and CO_2 concentration on spikelet sterility in indica rice. *Field Crops Res.* **51,** 213–219.

Matsui, T., Omasa, K., and Horie, T. (1997b). High temperature-induced spikelet sterility of *japonica* rice at flowering in relation to air temperature, humidity and wind velocity conditions. *Jpn. J. Crop Sci.* **66,** 449–455.

Matsui, T., Omasa, K., and Horie, T. (1999). Mechanism of anther dehiscence in rice (*Oryza sativa* L.). *Ann. Bot.* **84,** 501–506.

Matsui, T., Omasa, K., and Horie, T. (2000). High temperature at flowering inhibits swelling of pollen grains, a driving force for thecae dehiscence in rice (*Oryza sativa* L.). *Plant Prod. Sci.* **3,** 430–434.

Matsui, T., Omasa, K., and Horie, T. (2001). The difference in sterility due to high temperatures during the flowering period among *japonica*-rice varieties. *Plant Prod. Sci.* **4,** 90–93.

Matsui, T., Kobayasi, K., Kagata, H., and Horie, T. (2005). Correlation between viability of pollination and length of basal dehiscence of the theca in rice under a hot-and-humid condition. *Plant Prod. Sci.* **8,** 109–114.

Matsushima, S., and Tsunoda, K. (1958). Analysis of developmental factors determining yield and its application to yield prediction and culture improvement of lowland rice. XLV. Effects of temperature and its daily range in different growth-stages upon the growth, grain yield and its constitutional factors in rice plants. *Jpn. J. Crop Sci.* **26,** 243–244.

Matsushima, S., Tanaka, T., and Hoshino, T. (1964a). Analysis of yield-determining process and its application to yield-prediction and culture improvement of lowland rice. LXVIII. On the relation between morphological characteristics and photosynthetic efficiency. *Jpn. J. Crop Sci.* **33,** 44–48.

Matsushima, S., Tanaka, T., and Hoshino, T. (1964b). Analysis of yield-determining process and its application to yield-prediction and culture improvement of lowland rice. LXX. Combined effects of air-temperatures and water-temperatures at different stages of growth on the grain yield and its components of lowland rice. *Proc. Crop Sci. Soc. Jpn.* **33,** 53–58.

Matsushima, S., Tanaka, T., and Hoshino, T. (1964c). Analysis of yield-determining process and its application to yield-prediction and culture improvement of lowland rice. LXXI. Combined effects of air-temperatures and water-temperatures at different stage of growth on the growth and morphological characteristics of rice plants. *Proc. Crop Sci. Soc. Jpn.* **33,** 135–140.

Matsushima, S., Ikewada, H., Maeda, A., Honda, S., and Niki, H. (1982). Studies on rice cultivation in the tropics. I. Yielding and ripening responses of the rice plant to the extremely hot and dry climate in Sudan. *Jpn. J. Trop. Agric.* **26,** 19–25.

Matthews, R. B., and Stephens, W. (2002). Crop-Soil Simulation Models: Application in Developing Countries CABI Publishing, Wallingford, United Kingdom, 304pp.

Matthews, R. B., and Wassmann, R. (2003). Modelling the impacts of climate change and methane emission reductions on rice production: A review. *Eur. J. Agron.* **19,** 573–598.

Meullenet, J. F., Marks, B. P., Hankins, J. A., Griffin, V. K., and Daniels, M. J. (2000). Sensory quality of cooked long-grain rice as affected by rough rice moisture content, storage temperature, and storage duration. *Cereal Chem.* **77,** 259–263.

Mohammed, A. R., and Tarpley, L. (2009a). High nighttime temperatures affect rice productivity through altered pollen germination and spikelet fertility. *Agric. For. Meteorol.* **149,** 999–1008.

Mohammed, A. R., and Tarpley, L. (2009b). Impact of high nighttime temperature on respiration, membrane stability, antioxidant capacity, and yield of rice plants. *Crop Sci.* **49,** 313–322.

Moorman, F. R., and van Breemen, N. (1978). Rice: Soil, Water, Land. International Rice Research Institute, Los Banos, Philippines.

Morita, S. (2008). Prospect for developing measures to prevent high-temperature damage to rice grain ripening. *Jpn. J. Crop Sci.* **77,** 1–12.

Morita, S., Shiratsuchi, H., Takanashi, J.-I., and Fujita, K. (2004). Effect of high temperature on grain ripening in rice plants: Analysis of the effects of high night and high day temperatures applied to the panicle and other parts of the plant. *Jpn. J. Crop Sci.* **73,** 77–83.

Morita, S., Yonemaru, J. I., and Takanashi, J. I. (2005). Grain growth and endosperm cell size under high night temperatures in rice (*Oryza sativa* L.). *Ann. Bot.* **95,** 695–701.

Morokuma, M., and Yasuda, S. (2004). Effects of high temperature and high humidity during the flowering period on spikelet fertility in *japonica* rice. *Jpn. J. Crop Sci.* **73,** 93–98.

Mowjood, M. I. M., Ishiguro, K., and Kasubuchi, T. (1997). Effect of convection in ponded water on the thermal regime of a paddy field. *Soil Sci.* **162,** 583–587.

Murakami, T., Matsuba, S., Funatsuki, H., Kawaguchi, K., Saruyama, H., Tanida, M., and Sato, Y. (2004). Over-expression of a small heat shock protein, sHSP17.7, confers both heat tolerance and UV-B resistance to rice plants. *Mol. Breed.* **13,** 165–175.

Murata, Y. (1976). Productivity of rice in different climatic regions of Japan. "Climate and Rice", pp. 449–470. International Rice Research Institute, Los Banos, Philippines.

Nagai, T., and Makino, A. (2009). Differences between rice and wheat in temperature responses of photosynthesis and plant growth. *Plant Cell Physiol.* **50,** 744–755.

Nagarajan, S., Jagadish, S. V. K., Hari Prasad, A. S., Thomar, A. K., Anand, A., Pal, M., and Agarwal, P. K. (2010). Local climate affects growth, yield and grain quality of aromatic and non-aromatic rice in northwestern India. *Agric. Ecosyst. Environ.* **138,** 274–281.

Nagata, K., Takita, T., Yoshinaga, S., Terashima, K., and Fukuda, A. (2004). Effect of air temperature during the early grain-filling stage on grain fissuring in rice. *Jpn. J. Crop Sci.* **73,** 336–342.

Nagato, K., Ebata, M., and Kono, Y. (1961). On the adaptability of rice varieties to high temperature in the ripening periods. *Jpn. J. Crop Sci.* **29,** 337–340.

Nagato, K., Ebata, M., and Kishi, Y. (1966). Effect of high temperature during ripening period on the qualities on *indica* rice. *Proc. Crop Sci. Soc. Jpn.* **35,** 239–244.

Nakagawa, H., Horie, T., Kim, H. Y., Ohnishi, H., and Homma, K. (1997). Rice responses to elevated CO_2 concentration and high temperatures. *J. Agric. Meteorol.* **52,** 797–800.

Neue, H. U., Becker-Heidmann, P., and Scharpenseel, H. W. (1990). Organic matter dynamics, soil properties and cultural practices in rice lands and their relationship to methane production. *In* "Soils and Greenhouse Effect" (A. F. Bouwman, Ed.), pp. 457–466. John Wiley & Sons, New York.

Neue, H. U., Gaunt, J. L., Wang, Z. P., Becker-Heidmann, P., and Quijano, C. (1997). Carbon in tropical wetlands. *Geoderma* **79,** 163–185.

Newman, Y. C., Sollenberger, L. E., Boote, K. J., Allen, L. H. Jr., and Littell, R. C. (2001). Carbon dioxide and temperature effects on forage dry matter production. *Crop Sci.* **41,** 399–406.

Nijs, I., Ferris, R., Blume, H., Hendry, G., and Impens, I. (1997). Stomatal regulation in a changing climate: A field study using Free Air Temperature Increase (FATI) and Free air CO_2 enrichment (FACE). *Plant Cell Environ.* **20,** 1041–1050.

Nishiyama, I. (1976). Effects of temperature on the vegetative growth of rice plants. "Climate and Rice", pp. 159–185. International Rice Research Institute, Los Banos, Philippines.

Nishiyama, I. (1977). Decrease in germination activity of rice seeds due to excessive desiccation in storage. *Jpn. J. Crop. Sci.* **46,** 111–118.

Nishiyama, I., and Blanco, L. (1980). Avoidance of high temperature sterility by flower opening in the early morning. *Jpn. Agric. Res. Q.* **14,** 116–117.

Nishiyama, I., and Satake, T. (1981). High temperature damages in rice plants. *Jpn. J. Trop. Agric.* **25,** 14–19.

Nishiyama, I., Ito, N., Hayase, H., and Satake, T. (1969). Protecting effect of temperature and depth of irrigation water from sterility caused by cooling treatment at the meiotic stage of rice plants. *Proc. Crop Sci. Soc. Jpn.* **38,** 554–555.

Norby, R. J., Edwards, N. T., Riggs, J. S., Abner, C. H., Wullschleger, S. D., and Gunderson, C. A. (1997). Temperature-controlled open-top chambers for global change research. *Glob. Change Biol.* **3,** 259–267.

O'Toole, J. C. (1982). Adaptation of rice to drought-prone environments. "Drought Resistance in Crops with Emphasis on Rice", pp. 195–216. International Rice Research Institute, Manila, Philippines.

Oh-e, I., Saitoh, K., and Kuroda, T. (2007). Effects of high temperature on growth, yield and dry-matter production of rice grown in the paddy field. *Plant Prod. Sci.* **10,** 412–422.
Ohta, S., and Kimura, A. (2007). Impacts of climate changes on the temperature of paddy waters and suitable land for rice cultivation in Japan. *Agric. For. Meteorol.* **147,** 186–198.
Okada, M., Hamasaki, T., and Hayashi, T. (1995). Temperature gradient chambers for research on global environment change. I. Thermal environment in a large chamber. *Biotronics* **24,** 85–97.
Osada, A., Sasiprada, V., Rahong, M., Dhammanuvong, S., and Chakrabandhu, M. (1973). Abnormal occurrence of empty grains of *indica* rice plants in the dry, hot season in Thailand. *Proc. Crop Sci. Jpn.* **42,** 103–109.
Owen, P. C. (1971). The effects of temperature on the growth and development of rice. *Field Crop Abstr.* **24,** 1–8.
Paembonan, S. A., Hagihara, A., and Hozumi, K. (1992). Long-term respiration in relation to growth and maintenance processes of the aboveground parts of a *hinoki* forest tree. *Tree Physiol.* **10,** 101–110.
Parashar, D. C., Gupta, P. K., Rai, J., Sharma, R. C., and Singh, N. (1993). Effect of soil temperature on methane emission from paddy fields. *Chemosphere* **26,** 247–250.
Pareek, A., Singla, S. L., and Grover, A. (1997). Short-term salinity and high temperature stress-associated ultrastructural alterations in young leaf cells of *Oryza sativa* L.. *Ann. Bot.* **80,** 629–639.
Patindol, J., Wang, Y. J., and Jane, J. I. (2005). Structure-functionality changes in starch following rough rice storage. *Starch Starke* **57,** 197–207.
Patrick, W. H. Jr. (1981). The role of inorganic redox systems in cortrolling reduction in paddy soils. "Proceedings of Symposium on Paddy Soil", pp. 107–117. Institute of Soil Science, Academia Sinica, Science Press, Beijing and Springer-Verlag, Berlin.
Peet, M. M., Sato, S., and Gardner, R. G. (1998). Comparing heat stress effects on male-fertile and male-sterile tomatoes. *Plant Cell Environ.* **21,** 225–231.
Peng, S., Huang, J., Sheehy, J. E., Laza, R. C., Visperas, R. M., Zhong, X., Centeno, G. S., Khush, G. S., and Cassman, K. G. (2004). Rice yields decline with higher night temperature from global warming. *Proc. Natl. Acad. Sci. USA* **101,** 9971–9975.
Penning de Vries, F. W. T. (1975). The costs of maintenance processes in plant cells. *Ann. Bot.* **39,** 77–92.
Penning de Vries, F. W. T., Jansen, D. M., Bergeten, H. F. M., and Bakema, A. H. (1989). Simulation of Ecophysiological Processes of Growth in Several Annual Crops. Purdoc, Wageningen, Netherlands, 271pp.
Pingali, P. L., Hossain, M., and Gerpacio, R. V. (1997). Asian Rice Bowls: The Returning Crisis? CAB International, Wallingford, UK, 341pp.
Ponnamperuma, F. N. (1965). Dynamic aspects of flooded soils and the nutrition of the rice plant. *In* "The Mineral Nutrition of the Rice Plant" (A. Tanaka., F. N. Ponnamperuma, H. R. von Uexkull and R. P. Humbert, Eds.), pp. 295–328. The Johns Hopkins Press, Baltimore, MD..
Ponnamperuma, F. N. (1972). The chemistry of submerged soils. *Adv. Agron.* **24,** 29–96.
Porter, J. R. (2005). Rising temperatures are likely to reduce crop yields. *Nature* **436,** 174.
Prasad, P. V. V., Boote, K. J., Allen, L. H. Jr., Sheehy, J. E., and Thomas, J. M. G. (2006). Species, ecotype and cultivar differences in spikelet fertility and harvest index of rice in response to high temperature stress. *Field Crops Res.* **95,** 398–411.
Pregitzer, K. S., and King, J. S. (2005). Effects of soil temperature on nutrient uptake. *In* "Nutrient acquisition by plants-An ecological perspective" (H. BassiriRad, Ed.), pp. 277–310. Springer-Verlag, Berlin and Heidelberg, Germany.
Ramakrishnan, B., Lueders, T., Dunfield, P. F., Conrad, R., and Friedrich, M. W. (2001). Archaeal community structures in rice soils from different geographical regions before and after initiation of methane production. *FEMS Microbiol. Ecol.* **37,** 175–186.

Rath, A. K., Ramakrishnan, B., and Sethunathan, N. (2002). Temperature dependence of methane production in tropical rice soils. *Geomicrobiol. J.* **19,** 581–592.
Reddy, K. R., Hodges, H. F., and McKinion, J. M. (1997). Crop modeling and applications: A cotton example. *Adv. Agron.* **59,** 225–290.
Reddy, K. R., Hodges, H. F., Read, J. J., McKinion, J. M., Baker, J. T., Tarpley, L., and Reddy, V. R. (2001). Soil-plant-atmosphere-research (SPAR) facility: A tool for plant research and modeling. *Biotronics* **30,** 27–50.
Resurreccion, A. P., Hara, T., Juliano, B. O., and Yoshida, S. (1977). Effect of temperature during ripening on grain quality of rice. *Soil Sci. Plant Nutr.* **23,** 109–112.
Ritchie, J. T. (1993). Genetic specific data for crop modelling. *In* "Systems Approaches for Agricultural Development" (F. W. T. Penning de Vries, P. S. Teng, and K. Metselaar, Eds.), pp. 77–93. Kluwer Academic Publishers, Dordrecht.
Roberts, E. H. (1965). Dormancy in rice seed. IV. Varietal responses to storage and germination temperatures. *J. Exp. Bot.* **16,** 341–349.
Roberts, E. H. (1972). Storage environment and control of viability. *In* "Viability of Seed" (E. H. Roberts, Ed.), pp. 14–58. Syracuse University Press, Syracuse, New York.
Rosenzweig, C., and Parry, M. L. (1994). Potential impact of climate change on world food supply. *Nature* **367,** 133–138.
Rowland-Bamford, A. J., Baker, J. T., Allen, L. H. Jr., and Bowes, G. (1996). Interactions of CO_2 enrichment and temperature on carbohydrate accumulation and partitioning in rice. *Environ. Exp. Bot.* **36,** 111–124.
Roy, M., and Ghosh, B. (1996). Polyamines, both common and uncommon, under heat stress in rice (*Oryza sativa*) callus. *Physiol. Plant.* **98,** 196–200.
Rustad, L. E., Campbell, J. L., Marion, G. M., Norby, R. J., Mitchell, M. J., Hartley, A. E., Cornelissen, J. H. C., and Gurevitch, J.GCTE-NEWS. (2001). A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. *Oecologia* **126,** 543–562.
Ryan, M. G. (1991). Effects of climate change on plant respiration. *Ecol. Appl.* **1,** 157–167.
Sahi, C., Agarwal, M., Singh, A., and Grover, A. (2007). Molecular characterization of a novel isoform of rice (*Oryza sativa* L.) glycine rich-RNA binding protein and evidence for its involvement in high temperature stress response. *Plant Sci.* **173,** 144–155.
Sakai, H., Hasegawa, T., and Kobayashi, K. (2006). Enhancement of rice canopy carbon gain by elevated CO_2 is sensitive to growth stage and leaf nitrogen concentration. *New Phytol.* **170,** 321–332.
Sasaki, O. (1992). Effect of high water-temperature on the morphology of crown roots and leaf in rice plants. *Jpn. J. Crop Sci.* **61,** 388–393.
Sasaki, O. (2002). Influence of high water temperature on leaf growth in rice plant (*Oryza sativa* L.). *Jpn. J. Crop Sci.* **71,** 198–205.
Sasaki, H., Hara, T., Ito, S., Uehara, N., Kim, H. Y., Lieffering, M., Okada, M., and Kobayashi, K. (2007). Effect of free-air CO_2 enrichment on the storage of carbohydrate fixed at different stages in rice (*Oryza sativa* L.). *Field Crops Res* **100,** 24–31.
Saseendran, A. S. K., Singh, K. K., Rathore, L. S., Singh, S. V., and Sinha, S. K. (2000). Effects of climate change on rice production in the tropical humid climate of Kerala, India. *Climate Change* **44,** 495–514.
Satake, T., and Yoshida, S. (1978). High temperature induced sterility in *indica* rices at flowering. *Jpn. J. Crop Sci.* **47,** 6–17.
Sato, K. (1972). Growth responses of rice plant to environmental conditions. I. The effects of air-temperatures on the growth at vegetative stage. *Jpn. J. Crop Sci.* **41,** 388–393.
Sato, K. (1979). High temperature damage to ripening in rice plant. *Jpn. Agric. Res. Q.* **13,** 90–95.

Sato, K., and Inaba, K. (1976a). High temperature injury of ripening in rice plant. V. On the early decline of assimilate storing ability of grains at high temperature. *Proc. Crop Sci. Soc. Jpn.* **45,** 156–161.

Sato, K., and Inaba, K. (1976b). High temperature injury of ripening in rice plant. IV. Effect of high temperature on ^{14}C-assimilation and translocation at an early ripening period. *Proc. Crop Sci. Soc. Jpn.* **45,** 151–155.

Sato, K., Inaba, K., and Tozawa, M. (1973). High temperature injury of ripening in rice plant. I. The effects of high temperature treatments at different stages of panicle development on the ripening. *Proc. Crop Sci. Soc. Jpn.* **42,** 207–213.

Sato, S., Kamiyama, M., Iwata, T., Makita, N., Furukawa, H., and Ikeda, H. (2006). Moderate increase of mean daily temperature adversely affects fruit set of *Lycopersicon esculentum* by disrupting specific physiological process in male reproductive development. *Ann. Bot.* **97,** 731–738.

Semenov, M. A., and Halford, N. G. (2009). Identifying target traits and molecular mechanisms for wheat breeding under a changing climate. *J. Exp. Bot.* **60,** 2791–2804.

Sethunathan, N., Rao, V. R., Adhya, T. K., and Raghu, K. (1983). Microbiology of rice soils. *CRC Crit. Rev. Microbiol.* **10,** 125–172.

Sharkey, T. D. (1985). Photosynthesis in intact leaves of C_3 plants: Physics, physiology and rate limitations. *Bot. Rev.* **51,** 53–105.

Sheehy, J. E., Dionora, M. J. A., and Mitchell, P. L. (2001). Spikelet numbers, sink size and potential yield in rice. *Fields Crop Res.* **71,** 77–85.

Sheehy, J. E., Mitchell, P. L., and Ferrer, A. B. (2006). Decline in rice grain yields with temperature: Models and correlations can give different estimates. *Field Crops Res.* **98,** 151–156.

Shi, C. L., Jin, Z. Q., Tang, R. S., and Zheng, J. C. (2007). A model to simulate high temperature-induced sterility of rice. *Chin. J. Rice Sci.* **21,** 220–222.

Shi, C. L., Jin, Z. Q., Zheng, J. C., and Tang, R. S. (2008). Effect of high temperature at meiosis stage on seed-setting rate in rice. *Acta. Agron. Sin.* **34,** 627–631.

Shimazaki, Y., Satake, T., Ito, N., Doi, Y., and Watanabe, K. (1964). Sterile spikelets in rice plants induced by low temperature during the booting stage. *Res. Bull. Hokkaido Natl. Agr. Exp. Stat. (Jpn.)* **83,** 1–9.

Shimono, H., Hasegawa, T., and Iwama, K. (2002). Response of growth and grain yield in paddy rice to cool water at different growth stages. *Field Crops Res.* **73,** 67–79.

Shimono, H., Hasegawa, T., Fujimura, S., and Iwama, K. (2004). Responses of leaf photosynthesis and plant water status in rice to low water temperature at different growth stages. *Field Crops Res.* **89,** 71–83.

Siddique, M. R. B., Hamid, A., and Islam, M. S. (1999). Drought stress effects on photosynthetic rate and leaf gas exchange of wheat. *Bot. Bull. Acad. Sin.* **40,** 141–145.

Siebenmorgen, T. J., and Cooper, N. T. W. (2006). Rice kernel properties. *In* "Encyclopedia of Agricultural, Food, and Biological Engineering" (D. R. Heldman, Ed.). Taylor & Francis, London, United Kingdompp. 1(1–4).

Siebenmorgen, T. J., Nehus, Z. T., and Archer, T. R. (1998). Milled rice breakage due to environmental conditions. *Cereal Chem.* **75,** 149–152.

Sinclair, T. R., Allen, L. H. Jr., and Drake, G. M. (1995). Temperature gradient chambers for research on global environment change. II. Design for plot studies. *Biotronics* **24,** 99–108.

Singh, R. P., Prasad, P. V. V., Sunita, K., Giri, S. N., and Reddy, K. R. (2007). Influence of high temperature and breeding for heat tolerance in cotton: A review. *Adv. Agron.* **93,** 313–385.

Singla, S. L., and Grover, A. (1993). Antibodies raised against yeast HSP104 cross-react with a heat- and abscisic acid-regulated polypeptide in rice. *Plant Mol. Biol.* **22,** 1177–1180.

Singla, S. L., Pareek, A., and Grover, A. (1997). High temperature. *In* "Plant Ecophysiology" (M. N. V. Prasad, Ed.), pp. 101–127. John Wiley, New York.

Sohn, S. O., and Back, K. (2007). Transgenic rice tolerant to high temperature with elevated contents of dienoic fatty acids. *Biol. Plant.* **51,** 340–342.

Stanhill, G. (2001). The growth of climate change science: A scientometric study. *Climate Change* **48,** 515–524.

Sullivan, C. Y. (1972). Mechanisms of heat and drought resistance in grain sorghum and methods of measurement. *In* "Sorghum in the Seventies" (N. G. P. Rao and L. R. House, Eds.), pp. 247–264. Oxford and IPH publishing Co., New Delhi, India.

Sun, H., and Siebenmorgen, T. J. (1993). Milling characteristics of various rough rice kernel thickness fractions. *Cereal Chem.* **70,** 727–733.

Sung, D.-Y., Kaplan, F., Lee, K.-J., and Guy, C. L. (2003). Acquired tolerance to temperature extremes. *Trends Plant Sci.* **8,** 179–187.

Takahashi, N. (1961). The relation of water absorption to germination of rice seed. *Sci. Rep. Res. Inst. Tohoku Univ. D* **12,** 61–69.

Tanaka, K., Onishi, R., Miyazaki, M., Ishibashi, Y., Yuasa, T., and Inoue, M. I. (2009). Changes in NMR relaxation of rice grains, kernel quality and physicochemical properties in response to a high temperature after flowering in heat-tolerant and heat-sensitive rice cultivars. *Plant Prod. Sci.* **12,** 185–192.

Tang, R. S., Zheng, J. C., Jin, Z.-Q., Zhang, D. D., Huang, Y. H., and Chen, L. G. (2008). Possible correlation between high temperature-induced floret sterility and endogenous levels of IAA, GAs and ABA in rice (*Oryza sativa* L.). *Plant Growth Regul.* **54,** 37–43.

Tao, L. X., Tan, H. J., Wang, X., Cao, L. Y., Song, J., and Cheng, S. H. (2008). Effects of high-temperature stress on flowering and grain-setting characteristics of Guodao 6. *Acta Agron. Sin.* **34,** 609–674.

Tashiro, T., and Wardlaw, I. F. (1991a). The effect of high temperature on the accumulation of dry matter, carbon and nitrogen in the kernel of rice. *Aust. J. Plant Physiol.* **18,** 259–265.

Tashiro, T., and Wardlaw, I. F. (1991b). Effect of high temperature on kernel dimensions and the type and occurrence of kernel damage in rice. *Aus. J. Agri. Res.* **42,** 485–496.

Tewolde, H., Fernandez, C. J., and Erickson, C. A. (2006). Wheat cultivars adapted to post-heading high temperature stress. *J. Agron. Crop Sci.* **192,** 111–120.

Tian, X.-h, Matsui, T., Li, S. H., and Lin, J. C. (2007). High temperature stress on rice anthesis: Research progress and prospects. *Chin. J. Appl. Ecol.* **18,** 2632–2636.

Tsukaguchi, T., and Iida, Y. (2008). Effects of assimilate supply and high temperature during grain-filling period on the occurrence of various types of chalky kernels in rice plants (*Oryza sativa* L.). *Plant Prod. Sci.* **11,** 203–210.

Tsunoda, K., and Matsushima, S. (1962). Analysis of yield-determining process and its application to yield prediction and culture improvement of low land rice. LXII. Effects of irrigation water temperature under different water depths on the growth, grain yield and yield components of rice. *Proc. Crop Sci. Soc. Jpn.* **31,** 19–22.

Tsutsuki, K., and Ponnamperuma, F. N. (1987). Behavior of anaerobic decomposition products in submerged soils. *Soil Sci. Plant Nutr.* **33,** 13–33.

Uchida, A., Jagendorf, A. T., Hibino, T., Takabe, T., and Takabe, T. (2002). Effects of hydrogen peroxide and nitric oxide on both salt and heat stress tolerance in rice. *Plant Sci.* **163,** 515–523.

Umemoto, T., and Terashima, K. (2002). Activity of granule-bound starch synthase is an important determinant of amylose content in rice endosperm. *Funct. Plant Biol.* **29,** 1121–1124.

Unger, I. M., Motavalli, P. P., and Muzika, R. M. (2009). Changes in soil chemical properties with flooding: A field laboratory approach. *Agric. Ecosyst. Environ.* **131,** 105–110.

US Census Bureau, World POPClock Projection (2009). http://www.census.gov/main/www/popclock.html.

Vani, B., Saradhi, P. P., and Mohanty, P. (2001). Alteration in chloroplast structure and thylakoid membrane composition due to in vivo heat treatment of rice seedlings: Correlation with the functional changes. *J. Plant Physiol.* **158,** 583–592.

Venkateswarlu, B., and Visperas, R. M. (1987). Source-sink relationships in crop plants. *Int. Rice Res. Paper Ser.* **125,** 1–19.

Vu, J. C. V., Allen, L. H. Jr., Boote, K. J., and Bowes, G. (1997). Effects of levated CO_2 and temperature on photosynthesis and rubisco in rice and soybean. *Plant Cell Environ.* **20,** 68–76.

Vu, J. C. V., Allen, L. H. Jr., and Widodo, W. (2007). Leaf photosynthesis and Rubisco activity and kinetics of soybean, peanut, and rice grown under elevated atmospheric CO_2, supraoptimal air temperature, and soil water deficit. *Curr. Top. Plant Biol.* **7,** 27–41.

Wahid, A., Gelani, S., Ashraf, M., and Foolad, M. R. (2007). Heat tolerance in plants: An overview. *Environ. Exp. Bot.* **61,** 199–223.

Wakamatsu, K. I., Sasaki, O., Uezono, I., and Tanaka, A. (2007). Effects of high air temperature during the ripening period on the grain quality of rice in warm regions of Japan. *Jpn. J. Crop Sci.* **76,** 71–78.

Wakamatsu, K. I., Sasaki, O., Uezono, I., and Tanaka, A. (2008). Effect of the amount of nitrogen application on occurrence of white-back kernels during ripening of rice under high-temperature conditions. *Jpn. J. Crop Sci.* **77,** 424–433.

Wang, C. H., Wan, S. Q., Xing, X. R., Zhang, L., and Han, X. G. (2006). Temperature and soil moisture interactively affected soil net N mineralization in temperate grassland in Northern China. *Soil Biol. Biochem.* **38,** 1101–1110.

Wassmann, R., Jagadish, S. V. K., Heuer, S., Ismail, A., Redona, E., Serraj, R., Singh, R. K., Howell, G., Pathak, H., and Sumfleth, K. (2009a). Climate change affecting rice production: The physiological and agronomic basis for possible adaptation strategies. *Adv. Agron.* **101,** 59–122.

Wassmann, R., Jagadish, S. V. K., Sumfleth, K., Pathak, H., Howell, G., Ismail, A., Serraj, R., Redoña, E., Singh, R. K., and Heuer, S. (2009b). Regional vulnerability of climate change impacts on Asian rice production and scope for adaptation. *Advn. Agron.* **102,** 91–133.

Watanabe, I., and Furusaka, C. (1980). Microbial ecology of flooded rice soils. *Adv. Microb. Ecol.* **4,** 125–168.

Weerakoon, W. M. W., Maruyama, A., and Ohba, K. (2008). Impact of humidity on temperature-induced grain sterility in rice (*Oryza sativa* L). *J. Agron. Crop Sci.* **194,** 135–140.

Wei, K. S., Cheng, F. M., Zhang, Q. F., and Liu, K. G. (2009a). Temperature stress at grain filling stage mediates expression of three isoform genes encoding starch branching enzymes in rice endosperm. *Rice Sci.* **16,** 187–193.

Wei, K. S., Zhang, Q. F., Cheng, F. M., Zhong, L. J., and Chen, N. (2009b). Expression profiles of rice soluble starch synthase isoform genes in response to high temperature. *Acta. Agron. Sin.* **35,** 18–24.

Welch, J. R., Vincent, J. R., Auffhammer, M., Moya, P. F., and Dobermann, A. (2010). Rice yields in tropical/subtropical Asia exhibit large but opposing sensitivities to minimum and maximum temperatures. *Proc. Natl. Acad. Sci. USA*, www.pnas.org/cgi/doi/10.1073/pnas.1001222107.

White, A., Cannell, M. G. R., and Friend, A. D. (2000). CO_2 stabilization, climate change and the terrestrial carbon sink. *Glob. Change Biol.* **6,** 817–833.

Wise, R. R., Olson, A. J., Schrader, S. M., and Sharkey, T. D. (2004). Electron transport is the functional limitation of photosynthesis in field-grown Pima cotton plants at high temperature. *Plant Cell Environ.* **27,** 717–724.

Wollenweber, B., Porter, J. R., and Schellberg, J. (2003). Lack of interaction between extreme high-temperature events at vegetative and reproductive growth stages in wheat. *J. Agron. Crop Sci.* **189,** 142–150.
Xie, X. J., Li, B. B., Li, Y. X., and Shen, S. H. (2009). High temperature harm at flowering in Yangtze River basin in recent 55 years. *Jiangsu J. Agric. Sci.* **25,** 28–32.
Xu, H. B., Wang, G. M., Wei, M., and Zhou, W. B. (2001). Correlation analysis of the characters of pollen grains and seed setting of rice under high temperature stress. *J. Southeast Agric. Univ.* **23,** 205–207.
Yamada, I., Hozumi, K., and Enomoto, C. (1955). On the artificial germination of pollen in rice plants: III. Temperature relations of pollen germination and pollen tube growth. *Jpn. J. Crop Sci.* **23,** 321.
Yamada, M., Hidaka, T., and Fukamachi, H. (1996). Heat tolerance in leaves of tropical fruit crops as measured by chlorophyll fluorescence. *Sci. Hortic.* **67,** 39–48.
Yamakawa, H., Hirose, T., Kuroda, M., and Yamaguchi, T. (2007). Comprehensive expression profiling of rice grain filling-related genes under high temperature using DNA microarray. *Plant Physiol.* **144,** 258–277.
Yamamoto, Y., Tamori, T., and Kawaguchi, S. (1985). Relations between weather and growth of rice plant. I. Effects of air-temperature on the growth of rice plant in the first half stage. *Bull. Toyama Agric. Exp. Stn.* **16,** 20–26.
Yan, C., Ding, Y., Liu, Z., Wamg, Q., Li, G., He, Y., and Wang, S. (2008). Temperature difference between the air and organs of rice plant and its relation to spikelet fertility. *Agric. Sci. Chin.* **7,** 678–685.
Yang, L. X., Huang, J. Y., Yang, H. J., Dong, G. C., Liu, G., Zhu, J. G., and Wang, Y. (2006). Seasonal changes in the effects of free-air CO_2 enrichment (FACE) on dry matter production and distribution of rice (*Oryza sativa* L.). *Field Crop Res.* **98,** 12–19.
Yin, Xinyou, Kropff, Martin J., and Ellis, Richard H. (1996). Rice flowering in response to diurnal temperature amplitude. *Field Crops Res.* **48,** 1–9.
Yoshida, S. (1973). Effects of temperature on growth of rice plant (*Oryza sativa* L) in a controlled environment. *Soil Sci. Plant Nutr.* **19,** 299–310.
Yoshida, S. (1976). Carbon dioxide and yield of rice. "Climate and Rice", pp. 211–221. International Rice Research Institute, Los Banos, Philippines.
Yoshida, S. (1978). Tropical Climate and Its Influence on Rice. IRRI Research Paper Series 20 International Rice Research Institute, Los Banos, Philippines.
Yoshida, S. (1981). Fundamentals of Rice Crop Science. International Rice Research Institute, Los Banos, Philippines.
Yoshida, S. (1983). Rice. *In* "Potential Productivity of Field Crops Under Different Environments" (W. H. Smith and S. J. Banta, Eds.), pp. 103–127. International Rice Research Institute, Los Banos, Philippines.
Yoshida, S., and Ahn, S. B. (1968). The accumulation process of carbohydrate in rice varieties in relation to their response to nitrogen in the tropics. *Soil Sci. Plant Nutr.* **14,** 153–161.
Yoshida, S., and Hara, T. (1977). Effects of air temperature and light on grain filling of an *indica* and *japonica* rice *(Oryza sativa* L.) under controlled environmental conditions. *Soil Sci. Plant Nutr.* **23,** 93–107.
Yoshida, S., and Parao, F. T. (1976). Climatic influence on yield and yield components of lowland rice in the tropics. "Climate and Rice", pp. 471–494. International Rice Research Institute, Los Banos, Philippines.
Yoshida, S., Satake, T., and Mackill, D. J. (1981). High-temperature stress in rice (Review). *IRRI Res. Paper Series* **67**, 5pp.
Yoshioka, Y., Iwata, H., Tabata, M., Ninomiya, S., and Ohsawa, R. (2007). Chalkiness in rice: Potential for evaluation with image analysis. *Crop Sci.* **47,** 2113–2120.

Zakaria, S., Matsuda, T., Tajima, S., and Niita, Y. (2002). Effect of high temperature at ripening stage on the reserve accumulation in seed in some rice cultivars. *Plant Prod. Sci.* **5,** 160–168.

Zhang, G. Z., Chen, L. Y., Zhang, S. T., Xiao, Y. H., He, Z. Z., and Lei, D. Y. (2006). Effect of high temperature stress on protective enzyme activities and membrane permeability of flag leaf in rice. *Acta Agron. Sin.* **32,** 1306–1310.

Zhang, G., Chen, L., Zhang, S., Zheng, H., and Liu, G. (2009). Effects of high temperature stress on microscopic and ultrastructural characteristics of mesophyll cells in flag leaves of rice. *Rice Sci.* **16,** 65–71.

Zheng, K. L., and Mackill, D. J. (1982). Effect of high temperature on anther dehiscence and pollination in rice. *SABRAO J.* **14,** 61–66.

Zheng, S. H., Nakamoto, H., Yoshikawa, K., Furuya, T., and Fukuyama, M. (2002). Influences of high night temperature on flowering and pod setting in soybean. *Plant Prod. Sci.* **5,** 215–218.

Zhou, Z., Robards, K., Helliwell, S., and Blanchard, C. (2010). Effect of storage temperature on rice thermal properties. *Food Res. Int.* **43,** 709–715.

Zhu, C. L., Xiao, Y. H., Wang, C. M., Jiang, L., Zhai, H. Q., and Wan, J. M. (2005). Mapping QTLs for heat tolerance during grainfilling in rice. *Chin. J. Rice Sci.* **19,** 117–121.

Zia, M. S., Salim, M., Aslam, M., Gill, M. A., and Rahmatullah (1994). Effect of low temperature of irrigation water on rice growth and nutrient uptake. *J. Agron. Crop Sci.* **173,** 22–31.

Ziska, L. H., and Manalo, P. A. (1996). Increasing night temperature can reduce seed set and potential yield of tropical rice. *Aust. J. Plant Physiol.* **23,** 791–794.

Ziska, L. H., Manalo, P. A., and Ordonez, R. A. (1996). Intraspecific variation in the response of rice (*Oryza sativa* L.) to increased CO_2 and temperature: Growth and yield response of 17 cultivars. *J. Exp. Bot.* **47,** 1353–1359.

CHAPTER FOUR

Aerobic Rice Systems

Rajendra Prasad*

Contents

Abstract

About 90% of rice is produced and consumed in Asia, where the demand for rice is on the increase due to increasing population. Rice is a semiaquatic plant and grows well under lowland flooded anaerobic conditions. Most high yielding varieties yielding 6–8 t/ha have been developed to suit such conditions. However, there are large areas, where rice is grown under upland aerobic conditions with drought tolerant varieties that yield about 1 t/ha or a little more. Aerobic rice varieties are now being developed that have drought tolerance as well as high yielding ability. Aerobic rice system (ARS) is a new production system in which rice is grown under nonpuddled, nonflooded, and nonsaturated soil

* Indian National Science Academy, New Delhi, India

Advances in Agronomy, Volume 111
ISSN 0065-2113, DOI: 10.1016/B978-0-12-387689-8.00003-5

conditions. The expected yields in ARS are somewhat lower than those obtained under lowland flooded conditions, but double or treble of that obtained under upland conditions. However, ARS has been successful in cool temperate regions. For warm humid rice growing regions, a partial aerobic rice system (PARS) may be a more plausible alternative. PARS technologies are available. Some available results, however, show a decline in rice yield over years in ARS and suggest that ARS may not be sustainable over a long period. The factors responsible appear to be N, P, K, Fe, and Zn deficiencies, weeds and soil-borne root-knot nematodes. Some of these problems can be overcome by introducing legume such as soybean in ARS/PARS. ARS/PARS call for application of herbicides and namaticides, which are going to add additional burden on poor Asian farmers. In addition, farmers have to be trained in the careful and proper use of herbicides and nematicides. Herbicides have also been associated with environmental pollution problems.

1. Introduction

1.1. Importance of rice in global food needs

Rice (*Oryza sativa* L.) was domesticated more than 7000 years ago in Assam-Meghalaya areas of India and the mountain regions of southeast Asia and southwest China (Swaminatham, 1984). Out of about 156 million hectares (Mha) under rice in the world, nearly 133 Mha are in Asia (FAI, 2009; IRRI, 2006). Asia produces about 540 million tons (Mt) out of the total global production of 660 Mt of rice. As regards other parts of the world, western and southern Africa has an area of about 8 Mha producing 11.6 Mt of rice (IRRI, 2006). Latin America and the Caribbean have an area of about 5.5 Mha under rice and produce about 26 Mt of grain (Pulver *et al*., 2010). Brazil has an area of 2.9 Mha under rice and produces 12.6 Mt of grain (Lafranco, 2010).

Rice is the most important staple food in Asia even today and provides 35–80% of total calorie intake (IRRI, 1997). Further, changing dietary preferences are also affecting rice consumption in other parts of the world, and rice demand is increasing at the rate of 6% each year in western and central Africa (Carriger and Vallee, 2007). Rice consumption is estimated at 581 Mt in 2015 as against a consumption of 531 Mt in 2005 (IRRI, 2006). To meet their demand, Africa imports about 10 Mt of rice each year (Mohapatra, 2010), and Latin America and the Caribbean have a deficit of 2 Mt rice each year (Pulver *et al*., 2010).

The cultivated area in Asia is not likely to increase; on the contrary, it may decrease due to increased demand for nonagricultural use, while the population in Asia is likely to increase to 3895 million in 2015 from 3522 million in 2005 (IRRI, 2006). Thus, most of the increase in rice production

has to come from improved varieties and agronomic technology. Rice production under current inputs and technology is likely to fail to meet the projected demand (Leeper, 2010), and there is an urgent need to increase rice productivity per hectare in the world. Increasing yields in aerobic rice systems (ARSs) can play a key role in increasing rice production globally.

1.2. Aerobic rice systems

In Asia, more than 50% of all water used for irrigation is for rice (Barker *et al.*, 1999). About 55% of the rice area is irrigated and accounts for 75% of the total rice production in the world (Bouman, 2001). Tuong and Bouman (2003) estimated that by 2025, 2 Mha of Asia's irrigated dry season rice and 13 Mha of its irrigated wetland rice may experience "physical water scarcity" and the rest of the approximately 22 Mha of irrigated dry season rice in South and Southeast rice may suffer from "economic water scarcity." Seasonal water inputs in lowland flooded rice may vary from 660 to 5280 mm depending upon soil texture and climatic conditions (Table 1). Efforts are therefore underway to develop water saving technologies such as alternate wetting and dry (AWD; Bouman and Tuong, 2001), continuous soil saturation (Borell *et al.*, 1997), irrigation at fixed soil moisture tensions varying from 0 to 40 kPa (Sharma *et al.*, 2002; Singh *et al.*, 2002), or irrigation at an interval of 1–5 days after disappearance of standing water (Chaudhary, 1997). Such water management systems are partial aerobic rice systems (PARSs). True ARS is a new production system in which rice is grown under nonpuddled, nonflooded, and nonsaturated soil conditions (Bouman, 2001; Tuong and Bouman, 2003). Thus in ARS, soils are kept aerobic almost throughout the rice growing season. In addition to lesser water availability, other factors in ARS include soil mechanical impedance,

Table 1 Typical seasonal water outflows and input in lowland rice

Item	Water outflow/input (mm)
Land preparation	160–1560
Crop growth period	
Evapotranspiration	
Wet season	400–500
Dry season	600–700
Seepage and percolation	
Heavy clays	100–500
Loamy/sandy soils	1500–3000
Total seasonal water input	660–5280

From Tuong and Bouman (2003).

increased oxygen supply to roots, accumulation of ethylene and carbon dioxide in root tissue and availability of nitrogen as nitrate in place of ammonium (the dominant N ion under flooded conditions), and a changed soil fauna (Sanchez, 1976; Vosenek and van der Veen, 1994).

1.3. Rice and anaerobic conditions

Rice (*O. sativa* L.) is one of few species known for their ability to germinate under anaerobic or anoxic conditions. Some other species that can germinate under anaerobic conditions are *Echinochloa* sp. or barnyard grass, the most prolific weed in rice fields (Barrett and Seaman, 1980), *Erythrina caffra*, *Nuphar luteum*, *Scirpus mucronatus*, and *Trapa natans* (the water chestnut) (Menegus *et al*., 1992). Of all these species, only *T. natans* is able to germinate by radical emergence. In rice and other species, only the shoot emerges under the anoxic conditions and the germinating seedling is without rootlet. The development of root is possible only if some oxygen is available, which is generally present in the surface water in the flooded field or in the atmosphere. After coming in contact with oxygen, the coleoptile allows the oxygen to penetrate through its hollow structure or through highly permeable aerenchyma (Kutschera *et al*., 1990). Additional aerenchyma may be induced by the action of ethylene in some cultivars (Justin and Armstrong, 1991). Also when the submerged rice coleoptile comes in contact with the oxygen, ethylene biosynthesis is encouraged (Pearce *et al*., 1992), which encourages its elongation (Ku *et al*., 1970). In addition to promoting elongation, ethylene may also reduce injury due to hydrogen peroxide by promoting the activity of ascorbic oxidase (Mehlhorn, 1992; Ushimaru *et al*., 1992). Thus in rice, not only root formation, but also leaf elongation is strongly inhibited in the absence of oxygen. This would explain longer and deeper roots in rainfed upland rice cultivars. For example, Angus *et al*. (1983) reported a maximum root length of 60 cm in upland rice cultivar C-171-136 as compared to 40 cm in lowland cultivar IR36. Kawata and Ishihara (1959) found that in aerobic soils, drying periods induced the development of root hairs and nodal roots. Better root growth in rice under AWD was also reported by Zhang *et al*. (2009) and Banoc *et al*. (2000). Thus although rice prefers an anaerobic environment, some oxygen is a must for successful rice production; truly speaking, it is a semiaquatic plant. This would explain high rice yields in irrigated rice ecosystem than in rainfed lowland, deep water, and tidal ecosystems, where oxygen is in short supply or in rainfed upland system, where water is in short supply. This would also explain why 55% of the irrigated rice ecosystem produces 75% of the world's rice. This necessitates and encourages more research on irrigated or aerobic rice. In general, rice is a water loving plant. As compared to other cereals, rice has thinner leaves, more stomata per unit leaf surface, thinner

cuticles in leaves as well as in panicles with lesser wax load (Bouman *et al.*, 2007; Lafitte and Bennet, 2002).

Despite its likeness for anaerobic environment, rice is a great adjuster to fluctuations in soil moisture, humidity, and temperature. That is why rice is grown at an altitude of 1500 m above sea level on Banaue rice terraces in the Cordilleras of Philippines as well as below sea level in the Kuttanad region of Kerala state of India. This tendency to adjust to such diverse environments has led to the evolution of a large number of species in rice. Mapping of rice genome (Goff *et al.*, 2002; Yu *et al.*, 2002) has helped in delineating different rice species. There are four well-defined species complexes in rice, namely, *O. sativa* ($2n = 24$; Genome AA) grown worldwide, *O. officinalis* ($2n = 24, 48$; Genomes BB, CC, BBCC, CCDD, EE) grown in South Africa, East Africa, South and Central America, and Tropical Australia, *Oryza meryerian* ($2n = 24$; Genome GG) grown in South and Southeast Asia, *Oryza ridleyi* ($2n = 48$; Genome HHJJ), and some unknown genomes ($2n = 24, 48$; Genome FF, unknown) (Khush, 2005). The Asian cultivated species *O. sativa* has differentiated into three ecogeographic races, namely, *indica* (India and adjoining countries), *japonica* or *sinica* (Japan and China), and *javanica* (Indonesia) (Swaminatham, 1984).

2. Developments of Aerobic Rice Varieties

2.1. Principles

Aerobic rice production aims at a separate target environment (TE) as compared to traditional upland rice environment, where yields are substantially low due to shortage of water on critical growth stages. Since most upland rice is rainfed (without irrigation), the emphasis in breeding programs is on traits that protect the crop from drought. Most conventional cultivars developed for upland TE are tall, have fewer tillers, and often produce low but stable yields under low fertility conditions. They tend to have low harvest indices and tend to lodge under high fertility. Breeding programs for aerobic rice focus on traits related to: (a) drought tolerance as well as (b) response to inputs like fertilizers (Atlin *et al.*, 2006). Plants can overcome drought either by tolerance or by escape (Levitt, 1980). Drought tolerance is the ability of plants to maintain high leaf water potential under reduced soil moisture potential (SMP) and avoid dehydration; true protoplasmic resistance. Root characteristics, such as, density, length, and thickness (Yadav *et al.*, 1997) and greater root penetration (Ali *et al.*, 2000; Clark *et al.*, 2000) are important for aerobic rice varieties (ARVs; Fukai and Cooper, 1995; Nguyen *et al.*, 1997). As regards protoplasmic resistance, osmotic adjustment (OA) is an important shoot related characteristic for drought tolerance. OA refers to active accumulation of solutes during the development of water stress in plants (Blum, 1988), which allows the

maintenance of higher turgor potential at a given leaf water potential. OA delays leaf rolling, tissue death, and leaf senescence under water stress (Hsiao *et al.*, 1984). Plants can escape drought by completing its life cycle well ahead of the drought (Ram *et al.*, 1996). An example of such rice varieties is Jaldi Dhan 13 (meaning quick or early rice) released for drought-prone areas in West Bengal, India (Yadav *et al.*, 2007). Simulation models for understanding the effect of drought on crop growth involving soil, climate, and plant factors have been suggested (Penning de Vries *et al.*, 1989; Woperies *et al.*, 1996). In recent years, significant progress has been made in research on molecular basis for drought stress tolerance and Wang *et al.* (2005) have suggested that 16 candidate genes could potentially be involved in drought stress response. Of course, it will take some time before suitable techniques based on this research are developed and employed in screening rice varieties for drought tolerance.

Most aerobic rice cultivars have been developed through crosses of traditional upland cultivars for traits for drought tolerance and improved lowland varieties for traits on high yields. Recent trends involve identification of molecular markers for different traits in rice and mapping of quantitative trait loci (QTLs) associated with water saving is important for MAS (Marker Assisted Selection) of desirable plants. QTLs have been detected for several root related traits and OA in rice (Ali *et al.*, 2000; Lilly and Ludlow, 1996; Ray *et al.*, 1996; Yadav *et al.*, 1997; Zhang *et al.*, 2001; Zheng *et al.*, 2000) and will help in breeding for such traits. Several QTLs mapped for root length are common among mapping populations (Price and Courtois, 1999). Further, recent advances in genomics of rice and genetic engineering is likely to speed up the development of high yielding rice varieties with desirable traits for aerobic conditions (Farooq *et al.*, 2009).

Aerobic rice cultivars may yield lesser than lowland cultivars under continuous flooding but higher than them under aerobic (near saturation) conditions. For example, in trials in Changping and Changle counties (near Beijing), China, an aerobic rice cultivar HD502 yielded only 6.8 t/ha as compared to 8.8 t/ha obtained with a lowland cultivar JD305 under continuous flooded conditions (Bouman *et al.*, 2002) (Table 2). However,

Table 2 Yield (t/ha) of aerobic (HD297, HD502) and lowland (JD305) rice cultivars under different moisture regimes at Changpin and Changle in China

Location	Soil moisture regime	JD305	HD297	HD502
Changle	Flooded throughout rice growth	8.8	5.4	6.8
Changpin 1234	80–90% saturation throughout rice growth	4.2	4.7	5.3
	Rainfed; life saving irrigation when crop showed drying up	1.2	2.5	3.0

From Bouman *et al.* (2002).

under near saturation conditions (80–90%) in soil, HD502 yielded 5.3 t/ha as against 4.2 t/ha obtained with JD305, an increase of 22% over JD305. Further, under rainfed conditions, HD502 gave an yield of 3 t/ha, which was two and half times of that obtained with JD305.

2.2. China

In China, the breeding program for aerobic rice was started in 1980s at the China Agricultural University (CAU), China Academy of Agricultural Sciences (CAAS), the Liaoning Province Academy of Agricultural Sciences (LPAAS), and the Dandong Academy of Agricultural Sciences (DAAS). During 1980s and 1990s varieties, such as, Qinai, Hedda 77-2, Zhougyuan 1 & 2 and Han 72 were bred and released. However, due to some shortcomings such as vulnerability to rice blast, weak ability to emerge through the soil surface and low vegetative vigor limited their adoption (Huaqi *et al.*, 2002). Later at CAU, a strong genetic recombination of lowland and upland rice varieties was started in 1984. This included upland varieties from Yunnan province of China, Thailand, and Laos and lowland varieties from China and Japan. Early generation material was grown in lowland environments for selection in plant architecture, while late generation material was grown in aerobic environments for drought tolerance. This led to the development of new generation of elite ARVs, such as, Han Dao (HD) 297 from Mujiao 78-595 (a lowland variety(LV)) + Khaoman (an upland variety(UV)), HD277 from Quiguang (LV) + Ban Li 1 (UVs) and HD502 from Quiguang (LV) + Hangkelaoshuya (UV). In addition, LPAA released Han 58 and DAAS released Danjing 5. These new varieties have stronger drought tolerance, reduced plant height, increased lodging resistance erect upper leaves stronger resistance to blast, higher grain yield, and better grain quality. HD277 and H58 are currently the most extensively grown ARVs in China (Huaqi *et al.*, 2002).

On lighter soils with high seepage and percolation rates, aerobic rice has an advantage and breeders in China have produced varieties with an estimated yield potential of 6–7 t/ha and these are being cultivated on about 190,000 ha (Wang *et al.*, 2002). In temperate region of China, yields up to 5.7 t/ha have been reported with aerobic rice on a sandy loam soil at a research station near Beijing (Bouman and van Laer, 2006), whereas in on-farm trials yield ranged from 4.5 to 6.5 t/ha (Bouman *et al.*, 2002).

2.3. Brazil

In Brazil, the work on breeding aerobic rice was done at National Research Centre for Rice and Beans (CNPAF) of Empresa Brasileira de Pesquisa Agropecuria (EMBRAPA) located in Goias (Pinheiro *et al.*, 2006). There have been three distinct phases. In phase I (1975–1985), the emphasis was on drought tolerance, blast resistance, and yield stability targeting exclusively the

unfavorable savannah region. In phase II (1985–1990), the breeding strategy expanded to include selection for high yield potential targeting the favorable savannah area. In phase III (1990 onward), the emphasis is on grain quality, yield potential, and blast resistance. The strong priority for blast resistance continues in the aerobic rice breeding program due to high incidence of blast in the savannah system (Prabhu *et al.*, 1999; Prabhu and Filippi, 2002). Rice blast caused by fungus *Pyricularia grisea* is an important pathogen in rice grown on acid soils especially in dry years with heavy nitrogen fertilization (Zeigler *et al.*, 1994). The genetic basis of improved disease resistance has been examined through QTL analysis (Wang *et al.*, 1994) and molecular methods to accentuate progress in disease resistance are emerging (Lafitte *et al.*, 2002). Grain quality including those after cooking (dry, fluffy, and nonsticky) characters were introduced through indica group (Guimaraes *et al.*, 2001b). Maravilha and Primavera were the first upland rice varieties (released in 1996) that combined the grain quality and desirable aerobic rice characteristics (Pinheiro, 1999). Primavera received preference rating very close to BR IRGA 409, considered commercially the most competitive irrigated variety (Guimaraes *et al.*, 2001b). Recent released ARVs are Talento and Soberana (Pinheiro *et al.*, 2006). These varieties are *japonica-indica* derivatives and the yield potential is 5 t/ha. A latest addition to rice varieties for uplands of Brazil is “AN Cambara” (Santana, 2010). The high input rice production system in Brazil has led to the development of input responsive cultivars combined with management practices that reduce the risk of production in aerobic soils including a shift to less drought-prone areas.

2.4. International Rice Research Institute, Philippines

In addition to its association with the development of ARVs in China, IRRI initiated its own program for developing ARVs with a focus on tropical and subtropical regions. A multinational varietal testing program including *indica, japonica, aus*, and intermediate types was launched in 1986 with focus on “genotype × environment” and blast resistance (Lafitte *et al.*, 2002; Mckill *et al.*, 1996). The first generation of tropical ARVs developed by IRRI includes IR55423-01 (named Apo) and UPLRI-5 from the Philippines, B6144-MR-6-0-0 from Indonesia and CT6510-24-1-2 from Columbia. These varieties are derived from crosses between *indica* and tropical *japonica* parents. Data on the performance of some of these varieties at IRRI are in Table 3. In the Philippines, Peng *et al.* (2006) and Bouman *et al.* (2005) reported yields up to 6 t/ha.

2.5. India

The work on developing varieties suitable for ARS started only recently and is generally restricted to screening available varieties. At the Indian Agricultural Research Institute (IARI), New Delhi, APO, IR55419-04,

Table 3 Performance of some upland rice genotypes in a range of seasons and management systems vis-à-vis IR72 a lowland genotype at IRRI

Cultivar/line	Description	Average yield (t/ha)	Country of origin
Azucena	Traditional *japonica*	1.4	Philippines
B6144	Improved *indica*	2.1	Indonesia
IR60080-46A	Improve *japonica*	2.2	Philippines
IRAT104	Improved *japonica*	1.8	Ivory coast
IR55423-01(Apo)	Improved *indica*	3.4	Philippines
IR555435-05	Improved *indica*	3.2	Philippines
IR72	Improved *indica* lowland	2.6	Philippines

From Lafitte *et al.* (2002).

IR7437-46-1-1 (IRRI varieties), Pusa 834, Pusa RH 10 (IARI varieties), and Pro-Agro 6111 (a commercial variety) yielded above 4 t/ha under aerobic conditions. The water productivity of these varieties ranged from 0.42 to 0.47 kg/m^3 with irrigation at 20 kPa SMP (soil moisture potential), while it was 0.50–0.55 kg/m^3 at 40 kPa SMP (Singh and Chinnusamy, 2007). Further in on-farm trials with irrigation around field capacity, yields of 4.75–5.75 t/ha were obtained with a number of high yielding varieties developed at IARI for lowland flooded conditions, namely, Pusa Hybrid 10 and Basmati types Pusa Sugandh (meaning smell) 3, Pusa Sugandh 4, and Pusa Sugandh 5, which fetch much higher returns (Singh and Chinnusamy, 2007).

At Coimbatore, 12 upland varieties were direct seeded with water soaked seeds and grown with 2.5 cm depth of irrigation during the first 30 days followed by 3 cm irrigation later. The total water used varied from 432 mm in PMK3 to 654 mm in ADT46. Variety PMK3 produced most productive tillers and gave the highest grain yield of 3.68 t/ha. PMK3 also gave the highest water productivity of 7.66 kg rice ha/mm (Martin *et al.*, 2007).

University of Agricultural Sciences, Bangalore, has developed an ARV MAS 946-1, while another genotype MAS 26 has been approved for multilocation trials in India (Hittalmani, 2009).

3. Water Saving Techniques

Rice needs about 1700–3000 l of water to produce 1 kg grain depending upon the water availability (rain + irrigation), soil type (texture, organic matter content, hydraulic conductivity, percolation rate, etc.), and climate (temperature, sunshine hours, humidity, wind velocity, etc.).

Table 4 Irrigation requirement of rice and components of water at Pantnagar (India) under continuous submergence in soils of different texture

Particulars	Clay loam	Silt loam	Loam	Sandy loam
Effective rainfall (mm)	358	402	495	485
Irrigation requirement (mm)	1125	1200	1500	1775
Total water requirement (TWR) (mm)	1566	1657	1955	2262
Percolation (% of TWR)	57.0	52.5	60.0	66.9
Evaporation ET (5 of TWR)	44.0	44.2	41.3	32.9

From Gupta *et al.* (2002).

Some data on the effect of soil texture on water needs of rice at Pantnagar, India are in Table 4, which show that the total water requirement of rice increased from 1566 mm in a sandy clay loam soil to 2262 mm in a sandy loam soil, mainly due to an increase in percolation loss from 57% in clay loam to 66.9% in sandy loam soil (Gupta *et al.*, 2002). However, day by day water is getting scarce (Gleik, 1993; Guerra *et al.*, 1998) and by 2025 only 50–55% of the total world water will be available for agriculture as against 66–68% in 1993 (Sivannapan, 2009a). In most irrigated rice regions, groundwater is the main source of supplemental irrigation over and above the rain and canal water. In irrigated rice culture, there is a tendency to overirrigate rice fields resulting in wastage of water. This results in lowering of water table, and in some regions this has reached an alarming situation. For example, in North China Plain (NCP), water table is declining by 1–3 m each year (Sha *et al.*, 2000), while in the Indo-Gangetic Plain (IGP) of India it is declining by 0.5–0.7 m each year (Carriger and Vallee, 2007; Tuong and Bouman, 2003). This is bound to happen because, in the Punjab state of IGP the water pumped out to irrigate rice, when expressed as percentage (%) of recharge was 169–350 (Prasad, 2010). Heavy competition for water between different states and different sectors (industry, household needs in cities) is causing water scarcity for agriculture in southern India's Cauvery delta and in Thailand's Chao Phraya delta (Postel, 1997).

Saving of water has received the attention of irrigation scientists in India and elsewhere since long time. Some of the techniques suggested are briefly discussed.

3.1. Shallow submergence throughout rice growth

Farmers generally tend to irrigate rice fields to provide a standing water column of 10–15 cm or more. However, 3–10 cm submergence was found to be sufficient for control of weeds and optimum rice yield (Batchelor and Roberts, 1983; Oelke and Mueller, 1969; Pandey and Mitra, 1971).

A shallow submergence gives all the same advantages as a higher standing water column, but reduces water need considerably. The major advantage of submergence is weed control. The risk of yield loss in direct seeded rice (DSR) due to weeds by all seeding methods is higher than for transplanted puddled rice (TPR) due to the suppression effect of standing water on germination and growth of weeds (Rao *et al.*, 2007). Bhagat *et al.* (1999) also observed that continuous shallow submergence up to panicle initiation reduced weed diversity, density, and biomass. For example, Singh *et al.* (2009) reported that in the first year of their study in Bihar, India, the broad leaf weeds were 144 m^{-2} in DSR as compared to 109 m^{-2} in TPR; the values for sedges were 365 and 128 for DSR and TPR, respectively. Further, the efficiency of some herbicides also improves with standing water. Hach *et al.* (1997) reported that increased flooding depth enhanced the efficiency of pyrazosulfuron ethyl. Similarly, Pretilachlor requires stagnation of water for a few days for its full efficiency (Singh *et al.*, 2009). The other advantages of shallow submergence include regulation of soil temperature (important in severely cold and hot regions), dissipation of excess solar energy and development of favorable microclimate (Dastane and Nelliat, 1970), and favorable growth of blue green algae (BGA; Swarnalakshmi *et al.*, 2006).

3.2. Alternate wetting and drying/partial aerobic rice systems

3.2.1. Withdrawing water at a growth stage

Dastane (1967) reported that withholding water for a period of 20 days during tillering or primordia development and flowering reduced rice grain yield by 16%, while withholding it for the same period during flowering to grain formation reduced the yield by 9% and withholding it during grain maturity reduced it by 3% only. Singh and Misra (1974) reported a reduction in rice yield of 19%, 29%, and 34% when water was in short supply at tillering, stem elongation, and panicle initiation stages, respectively. De Datta *et al.* (1975) reported that a water deficit during vegetative phase reduced rice yield by 34%, whereas water deficit during reproductive phase reduced it by 50%. According to Boonjung and Fukai (1996), the effect of water stress on rice yield was most severe when drought occurred during panicle development; anthesis was delayed and the number of spikelets per panicle and the percentage of filled grains was reduced. Rice is thus very sensitive to reduction in water availability around flowering as this results in spikelet sterility (Cruz and O'Toole, 1984; Ekanayke *et al.*, 1989). About 30% of the water transpired by rice after flowering is lost through the panicles (Bouman *et al.*, 2007). Recent studies in Senegal (Mendoza, 2010) indicate that the best results (yield cum water saving) were obtained when the rice field was kept flooded during the first half growing season (vegetative phase) and under AWD during the second phase (reproductive phase) of the growing period. Maintaining AWD throughout the growth

period of rice reduced the grain yield of rice by 20%. AWD is also gaining ground in Bangladesh (Tuong, 2009)

3.2.2. Withholding irrigation for a few days after disappearance of standing water

Tripathi (1992) reported that a submergence of 7.5 cm, 3 days after the soil dried, was the best water management practice for rice. In studies at a number of research stations under the All India Co-ordinated Research Project on Water Management (AICRPWM) of the Indian Council of Agricultural Research, providing irrigation 3 days after disappearance of standing water gave as good yields as under continuous submergence but brought about a saving of 40–54% in irrigation water (Chaudhary, 1997). Grigg *et al.* (2000) also indicated that the duration of flood water application currently practiced in southern USA can be reduced without sacrificing yield. Sharma (1989) observed that rice can withstand the water stress (about 10–15 kPa), which may be observed during 2–4 days after the disappearance of standing water.

3.2.3. Intermittent submergence and near saturation

In studies under AICPWM in India, a number of field experiments were conducted during 1970s to compare shallow submergence throughout the rice growth with intermittent submergence (shallow submergence during tillering and flowering stages and near saturation (5 cm) submergence to field capacity) at other stages and saturation throughout the rice growth. The rice yields obtained with intermittent submergence were 86–105%, while that with saturation were 71–102% of those obtained with shallow submergence throughout rice growth (Sharma, 1999; Yadav, 1972). However, these treatments considerably reduced the amount of irrigation water.

3.2.4. Conservation technologies

Inspired by the success of raised bed systems (RBSs) in wheat–maize cropping system in Mexico (Meisner *et al.*, 1992; Sayre and Hobbs, 2004), RBS was successfully tested in wheat in the IGP (Dhillon *et al.*, 2000). It was later extended to rice–wheat cropping system (RWCS; Connor *et al.*, 2002; Kukal *et al.*, 2008), which is the backbone of food security in India (Prasad, 2005). In such a study at New Delhi (Singh *et al.*, 2002) on a sandy loam soil, rice yield, irrigation water demand and evapotranspiration (ET) was the highest with continuously flooded TPR (Table 5). Direct seeding either on a wet bed (WSR) or a dry bed (DSR) gave only three-fourth of the grain yield obtained in TPR, while the reduction in irrigation water demand was 19% in WSR and 50% in DSR. The difference in WSR and DSR was due to large amount of water needed in making a wet bed (puddling). As compared to TPR, reduction in percolation loss was 8% in WSR and 43.7% in DSR. Direct seeding on flat or raised beds with SMP between saturation (00) to field capacity or 20 kPa gave only 56–58% of the grain yield obtained with TPR,

Table 5 Water balance and grain yield of rice as influenced by conservation technologies on a sandy loam soil at New Delhi

Treatment	Irrigation (mm)	ET (mm)	Percolation (mm)	Yield (t/ha)	WUE (g/kg water)	Yield (% of TPR)
Continuous flooding						
TPR	1360	781	828	5.5	0.34	–
WSR	1108	710	758	4.0	0.27	72.7
DSR	685	556	466	4.2	0.40	76.4
At different soil moisture potential (SMP)						
DSR-FB00	669	494	510	3.2	0.31	58.2
DSR-RB00	567	475	433	3.2	0.35	58.2
DSR-RB20	497	466	344	3.1	0.37	56.4
DSR-RB40	419	456	241	2.5	0.32	45.4

TPR, transplanted puddled rice; WSR, direct seeding on wet bed; DSR, direct seeding on dry bed; FB, flat bed; RB, raised bed; 00, saturated SMP; 20, SMP at 20 kPa (soil moisture content 8%, w/w); 40, SMP at 40 kPa (soil moisture content 6%, w/w).
From Singh *et al.* (2002).

although irrigation water need was considerably reduced. DSR on raised bed with irrigation at 40 kPa SMP gave the lowest grain yield of rice, which was only 45% of that obtained with TPR. Water use efficiency (WUE) for all but WSR varied from 0.32 to 0.40 g rice grain per kg water; WUE for WSR was 0.27 g rice grain per kg water. Thus AWD, in general, gave lower yield than TPR. Studies in USA have shown considerable saving in irrigation water with furrow irrigated raised beds (Tracey *et al.*, 1993; Vories *et al.*, 2002). When grown on raised beds, loss in cropping area due to wide furrows needs to be compensated by producing more productive tillers (Singh *et al.*, 2002).

Another conservation technology that helps in increasing WUE in rice is precision laser leveling (LL), a process of smoothing the land surface to a constant slope of 0–0.2% with the help of a large horse power tractor and soil movers equipped with laser guided instruments (Walkers *et al.*, 2003). In a study in India involving 16 on-farm trials, rice grain yield increased from 4.98 t/ha with traditional leveling (TL) to 5.41 t/ha with LL in transplanted puddled rice (TPR) and from 5.10 t/ha under TL to 5.25 under LL in DSR (Jat *et al.*, 2006). In this study, the water productivity (kg/m^3) increased from 0.331 under TL to 0.394 under LL in TPR and from 0.409 under TL to 0.468 under LL in DSR.

3.2.5. Microirrigation

The average overall efficiency of canal irrigation projects in rice growing areas of the world is estimated at a miserably low 23% (Walters and Bos, 1999). As a contrast, efficiency of trickle irrigation can approach 90–96%

(Bucks *et al.*, 1982), while that for sprinkler irrigation could be 80–90%. Minor irrigation (trickle/drip and sprinkler) can considerably economize on the water use and increase its efficiency. In India, it is so far restricted to high return crops such as fruits, vegetables, sugarcane, and cotton (Sivannapan, 2009b). In a study at the Water Technology Centre for Eastern region (WTCER), Bhubaneswar, India, the water required for piped conveyance was 152 mm as against 240 mm for surface irrigation, resulting in a saving of 36.6% water for the same or a slightly higher yield of rice (Srivastava, 2009). At IRRI, ARVs IR55423-01 (Apo) and Mangat performed very well under sprinkler irrigation. In Australia, sprinkler irrigation reduced water needs by 30–70% (Humphreys *et al.*, 1989), but even with frequencies up to three times per week, yield declined by 35–70% (Muirhead *et al.*, 1989). In Brazil, Stone *et al.* (1990) observed that sprinkler irrigation can be used in ARVs because of their high yields, which make it economically feasible. The ARVs are grown under rainfed conditions and are thus prone to drought and irrigation has to be applied when soil moisture in 0–15 cm surface soil layer reaches an SMP of 25 kPa.

In Australia, subsurface drip irrigation commencing 2 weeks prior to panicle initiation reduced irrigation water needs by 200 mm but resulted in decreased yield and there was no increase in water productivity (Beecher *et al.*, 2006). However, in India, on-farm trials conducted in the states of Tamil Nadu and Andhra Pradesh during 2009 and 2010 have shown encouraging results. The irrigation water need was reduced from 900 mm under continuously flooded conditions to 364 mm under drip method. Drip irrigation is presently highly subsidized in these states, 65% in Tamil Nadu and 90% in Andhra Pradesh (S. Das, The Financial Express, New Delhi, 3 July 2010).

3.3. Aerobic rice system

As already pointed out, ARS aims at growing rice without puddling and flooding under nonsaturated soil conditions as other upland crops. The driving force behind ARS is water economy and Castaneda *et al.* (2003) reported a saving of 73% in land preparation and 56% during crop growth. ARS heavily relies on herbicides and other biocides, such as, nematicides and adequate supply of plant nutrients including P, Fe, Zn, and others that may become deficient under aerobic conditions. Yields obtained with ARS varieties vary from 4.5 to 6.5 t/ha, which is about the double or treble of that obtained with traditional upland rainfed varieties and 20–30% lesser than that obtained with lowland varieties grown under flooded conditions (Farooq *et al.*, 2009). The major gain is saving in water, which may be 50–60% less in ARS as compared to transplanted puddled rice (TRP). ARS also requires lesser labor and can be mechanized (Belder *et al.*, 2004, 2005; Huaqi *et al.*, 2002). Based on their studies in NCP, Xue *et al.* (2008a) and

using ORYZA 2000 growth model predicted that rice grain yields of 6–6.8 t/ha can be reached under ARS with a total water input between 580 and 797 mm (rainfall 477 mm; irrigation 112–329 mm). For efficient water use, 2–4 irrigations were needed for maintaining SMP between 100–200 kPa in the root zone. The water productivity with respect to total water use was projected at 0.89–1.05 g grain/kg water. Models have been developed for water balance for ARS (Aggarwal *et al.*, 2004; Feng *et al.*, 2007; Luo *et al.*, 2006).

4. Sustainability of ARS/PARS

In aerobic rice experiments at the International Rice Research Institute (IRRI), yields of aerobic rice gradually declined over time as compared to a continuously flooded control (George *et al.*, 2002; Peng *et al.*, 2006). Some data are available from the experiments in India, where rice was grown on raised beds. At Modipuram, the relative yield of TPR on permanent beds declined progressively over the years, from 90% of permanent transplanted rice (PTR) in the first crop to 77% of PTR in the third rice crop (Singh *et al.*, 2005). From the same research centre, Jat *et al.* (2008) also reported that the yield of fourth crop of DSR on permanent beds was only 76% of PTR. Similarly in on-farm trials in Punjab, the yield of rice on raised beds is about half of that in PTR and the yield of both transplanted or DSR on beds declined substantially by the third rice crop (Kukal *et al.*, 2008).

Yield declines and even failures in continuous upland rice cultivation are reported from Philippines (George *et al.*, 2002; Ventura and Watanabe, 1978; Ventura *et al.*, 1984). In Brazil also, rice yield declined after 2 years of consecutive upland cultivation and after 5 years of monoculture, rice yield was only 1.5 t/ha as compared to 4.3 t/ha after 3 years of soybean (Fageria and Baligar, 2003; Pinheiro *et al.*, 2006).

Nishizawa *et al.* (1971) introduced the term "soil sickness" for the combined effect of allelopathy (Nishio and Kusano, 1975), nutrient depletion, built-up of soil-borne diseases and pests (Ventura *et al.*, 1981), and soil structure degradation. Thus, yield decline in ARS/PARS monoculture over time could be due to both abiotic and biotic stresses.

4.1. Soil abiotic stresses

4.1.1. Flooding and soil pH

Soil pH is an important characteristic and controls the availability of most essential plant nutrients and thereby the growth and yield of rice. Flooding overcomes both acid and alkaline (sodic) conditions in soil. For example,

Ponnamperuma (1972) reported that pH of an acid soil changed from 3.5 to near 6.0 and that of an alkaline soil changed from 8.1 to near 7.3 on submergence for a period of 2 weeks. One simple explanation is the dilution of H^+ or Na^+ ions. Ponnamperuma (1981) observed that for acid soils, the change in pH can be explained by the conversion of ferric to ferrous form, which is controlled by redox potential in soil. The redox potential required for the conversion of ferric to ferrous iron is $+180$ to $+150$ mv, while that for manganic to manganous Mn is $+280$ to $+220$ mv. The redox potential (Eh) for aerated (well drained) is $+700$ to $+500$ mv, while that for reduced soil is $+100$ to -100 mv (Patrick and Mahapatra, 1968). In highly reduced soils, the Eh is -100 to -300 mv. For calcareous soils, the partial pressure of CO_2 controls pH, while in calcareous alkaline soils, partial pressure of CO_2 as well as log (alkalinity) controls the pH (Ponnamperuma (1981)). In an aerobic rice study at Tarlan, Philippines, the pH of soil increased from 7.0 at seeding to near 8.0 at flowering in 2007 and was responsible for Mn deficiency (Kreye *et al.*, 2009a).

4.1.2. Soil acidity

A fairly large percentage of rice growing soils are Ultisols and Oxisols and are quite acidic. For a major part, flooding overcomes this problem and rice is successfully grown. However, in upland conditions soil acidity develops and a number of problems crop up, such as, Al-toxicity (Foy, 1992), phosphate fixation (Kirk *et al.*, 1998; Prasad and Power, 1997), and nutrient deficiencies (discussed individually). Oxisols are notorious for phosphorus immobilization because of their high iron oxide content (Smyth and Cravo, 1997).

Due to mediation of soil acidity by flooding, liming is generally not required and practiced in lowland rice. However, when an upland crop, such as, corn (*Zea mays*) is grown during wet season or wheat or a legume during dry season on acid soils, liming is required. In a long-term field experiment on an Alfisol (pH 5.3) at Ranchi, India, corn yield was zero after 14 years despite adequate NPK fertilization in the absence of lime but remained steady at about 3.7 t/ha when lime was applied (Nambiar, 1994). In the same experiment, wheat yield declined from 3.4 t/ha in 1970–1971 to 0.84 t/ha in 1983–1984 (a decline of 75%) when lime was not applied, but remained steady at 4 t/ha when lime was applied.

Lime requirement (LR) as determined by the method of Shoemaker *et al.* (1961) is generally very high and the poor Asian farmers can ill afford it. Also in such regions lime (calcite) is not available. An alternative cheap source to calcite is basic slag from the steel industry and has been suggested for liming (Ali and Shahram, 2007; Bhat *et al.*, 2007). In addition to high Ca content, basic slag also contains plenty of P, Zn, and Cu (Peregrina *et al.*, 2008). Data from long-term fertilizer experiments in India suggest that farmyard manure (FYM) or other organic manures can also mediate soil

acidity to some extent (Swarup, 2002). In a recent study (Bhat *et al.*, 2010) on rape/mustard (*Brassica compestris*)–rice cropping system (two crops a year rotation), basic slag and calcite were tried with or without FYM or poultry manure (PM). Lime was applied at 10% or 20% of SMP LR. All lime and manure was applied to rape/mustard and the succeeding rice was grown on residual lime. Application of lime as calcite or basic slag with or without manures increased soil pH from initial 5.1 to 5.9–6.1. The residual effects of lime applied to rape/mustard increased rice grain yield significantly and basic slag was better than calcite. Addition of manures further significantly increased rice grain yield over calcite or basic slag application. Application of calcite or basic slag left significantly more P, K, Ca, Zn, and Cu in soil than check after the harvest of rape/mustard; values for these nutrients were further significantly increased due to manures. The advantage of these higher values for nutrients in soil led to higher concentration of these nutrients in succeeding rice. Thus, rice does benefit from liming. These results also show that good results of liming can be obtained with much lesser amounts of lime. Addition of organic manures also helps crops in mediating harmful effects of soil acidity. Importance of lime in tropical rice soils has been recently reviewed (Fageria and Baligar, 2008). Sanchez (1976) reported that crops differ in their tolerance to exchangeable Al (ex. Al) in acid soils. He reported that crops such as rice, coffee, and pineapple can tolerate high levels of ex. Al and seldom respond to liming while corn is sensitive to 40–60% ex. Al and sorghum, cotton, and alfalfa can tolerate 10–20% ex. Al. Fageria (2000) reported that the availability of Fe and Mn decreased as the pH increased. However, the highest grain yield of rice was obtained at a pH of 6.4, indicating some toxicity of Fe and Mn at lower pH values. Liming acid soils can also reduce N_2O emission (Clough *et al.*, 2003).

Courtois and Lafitte (1999) observed that the best performance of a variety is recorded in the environment for which it was selected. Therefore, rice varieties bred under acid soil conditions perform well on acid soils; however, the differences do exist in their tolerance to soil acidity. For example, Fageria *et al.* (2004) reported that in Brazil the genotype CRO97505 yielded much higher than the other 5 genotypes (CNAs 8983, Primavera, Canastra, Bonaca, and Carisma) at pH 4.5 as well as at pH 6.4. As our understanding of the genetic basis of tolerance to acidity and low fertility in soil improves, mechanism based screening of these stresses may be possible. In addition to Ultisols and Oxisols, many rice growing countries have acid sulfate soils in the coastal areas and these soils have serious problems (Khan *et al.*, 2008). Results from a multiple correlation study indicated that A_{Fe} (the ratio of Fe^{2+} activity to the sum of the activity of all other divalent cations) and pH provided the best two variables in the model describing rice yield (Moore *et al.*, 1990). The other factors that affected rice yield were Al toxicity and P deficiency. ARS/PARS have not

yet been tried on such soils but Sahrawat (1979) reported that drying and reflooding an acid sulfate soil may aggravate soil acidity and keep iron in high amounts to be toxic to rice.

4.1.3. Soil salinity/sodicity (alkalinity)

Rice is less sensitive to salinity as compared to wheat and barley, yet yield reduction starts at an electric conductivity (EC) value of 3 dS/m; in wheat and barley, this happens at an EC value of 6 dS/m, respectively (Shannon, 1997). However, at an EC of 6 dS/m, the rice yield may decline by 50% and, at 10 dS/m, the decline my go up to 90%. Sensitivity of rice is most during early seedling and reproductive stages. Water management practices can affect salinity in rice fields (Scardeci *et al.*, 2002). Salinity is most widespread in coastal areas, while saline–sodic soils are more spread in inlands but also occur in coastal areas. The estimates of saline and/or sodic soils are at 9–12 Mha [6.7 Mha in India (Maji *et al.*, 2010); 1 Mha each in Bangladesh, Thailand, and Vietnam; and 1 Mha in Myanmar and Indonesia combined] (Bouman *et al.*, 2007). Considerable variation in rice varieties exists in sensitivity to salinity (Flowers and Yeo, 1981) and salinity tolerance has been introduced in high yielding types (Gregorio *et al.*, 2002). Central Soil Research Institute (CSSRA), Karnal, India has developed salinity/sodicity tolerant rice variety CSR5 and recently even a Basmati type CSR30 named as Yamini (Gautam *et al.*, 2009). The salinity tolerance can be improved by incorporating useful genes and or pyramiding superior alleles (Prasad *et al.*, 2000). Further, a recently mapped QTL designated "Saltol" can account for more than 70% of the variation in salt uptake (Bonilla *et al.*, 2002). The suitability of these new varieties is yet to be tested under aerobic conditions.

As regards sodicity, flooding reduces the concentration of Na^+ in soil solution and flooded rice does not suffer. In long-term experiments at Central Soil Salinity Research Institute, Karnal, India, growing rice for a period of 10 years made 1.2 m depth of soil nearly free of sodicity problem (Singh and Abrol, 1988), making it better for the succeeding wheat.

4.1.4. Nitrogen

AWD can lead to high N losses due to alternate nitrification (under dry conditions) and denitrification (under submerged conditions. In a laboratory study at New Delhi, about 95% of the N added as urea to soil was lost due to three cycles of submergence and drying in a period of 8 weeks (Prasad and Rajale, 1972). Aulakh *et al.* (2001) reported that 22–33% of applied fertilizer nitrogen to rice is lost due to denitrification. Pathak *et al.* (2005) using a simulation model approach predicted total production of N_2O/NO_x–N from rice fields in India (42 Mha) at 40–50 Gg/yr under continuous flooding and 50–60 Gg/yr under intermittent flooding. These values are much less as compared to estimates of global N_2O/NO_x emission of 22 Tg/yr (Mosier *et al.*, 2004). Thus, more N_2O/NO_x production under

ARS/PARS is another issue to be kept in mind. It may be mentioned that N_2O has about 300 times green house warming effects than CO_2 and has life time of about 166 years. Further, it is also involved in environmental ozone depletion (Grobecker *et al.*, 1975).

Under field conditions at New Delhi, an application of 150 kg N/ha as urea in two split applications under AWD gave 421 panicles/m^2, 77.5 spikelets/panicle and a grain yield of 5.6 t/ha as compared to 445 panicles^{-2}, 91 spikelets/panicle and a grain yield of 6.4 t/ha obtained under continuous flooding (Rajale and Prasad, 1975). Singh *et al.* (2002) reported that ammonium–N concentration in 0–15 cm soil layer at flowering was 135.1 kg/ha in continuously flooded TPR as compared to 51.7 kg/ha in DSR on raised beds at SMP of 40 kPa (DSR-RB40), while nitrate–N content was 34 kg/ha in TPR as compared to 63.9 kg/ha in DSR-RB40. The total ammonium + nitrate N was 169.2 kg/ha in TPR and 115.6 kg/ha in DSR-RB40. The trend was similar for 15–30 and 30–45 cm soil layers. The values for other AWD treatments were in between. Thus, AWD reduced N availability. A definite "nitrogen × irrigation" interaction exists, but the findings vary from place to place as would be expected. Studies at Ludhiana (India) indicated that application of N ameliorated the adverse effect of periodic moisture stress on rice yield; the reduction was 21% in the absence of N and only 5%, when 120 kg N/ha was applied (Aggarwal *et al.*, 1987). In studies in China, however, yields declined when heavy dressings of N were made to rice grown under AWD (50–70% of field capacity moisture, Xue *et al.*, 2008b). It may be mentioned that rates of application of N to rice in China are fairly high and may go up to 250 kg N/ha or more. Prasertsak and Fukai (1992) also reported that apart from increased risk of lodging, high rates of N application reduced growth and grain yield in rice. More studies on "nitrogen × irrigation" interaction are needed, so that definite recommendations are made to the AWD rice farmers.

Attempts are also being made to develop more N-efficient genetically modified (GM) rice plants. For example, Shrawat *et al.* (2008) genetically engineered a more N-efficient rice plant by introducing a barley AlaAT (alanine amintranferase)cDNA by a rice tissue-specific promoter (OsAnt1).

4.1.5. Phosphorus

The transformation and availability of native and applied P in soils depends upon a large number of factors, such as parent material, pH, organic matter, clay mineral composition, soil moisture, and the form in which it is applied (Prasad and Power, 1997; Vig *et al.*, 1999). The discussion here is restricted to the effect of soil moisture. The availability of native soil P generally increases on submergence due to the dissolution of occluded P (Patrick and Mahapatra, 1968), which of course varies from soil to soil (Mandal, 1979). Dobermann *et al.* (1998) pointed out that initial P flush due to submergence in rice paddies is generally followed by a decrease from the resorption or

precipitation of Fe–P compounds (Kirk *et al.*, 1998; Ponnamperuma, 1972). Drying a submerged soil decreases the availability of both native soil and applied P (Sah and Mikkelsen, 1989; Sah *et al.*, 1989a,b; Willet, 1982). Phosphorus deficiency in rice is difficult to diagnose and has been referred to as a hidden hunger (Dunn and Stevens, 2007). A good response to phosphorus is reported for rice (Shukla *et al.*, 2010). The best results are reported for hybrid rice grown on acid soils in India, where the yield of two successive crops of rice increased from 4.9 to 13.9 t/ha due to phosphate fertilization (Pattanayak *et al.*, 2008a). In RWCS, the general trend is to apply P to wheat and skip P application to rice (Prasad, 2005). However, a close examination of the data suggests that at least one-third of the total P to be applied to the system should be applied to rice (Prasad, 2007).

4.1.6. Potassium

A large number of rice soils, especially the Ultisols and Oxisols are deficient in K (Sekhon, 1999; Srinivasa Rao *et al.*, 2010), which are quite abundant in rice growing regions of the world and good response to K fertilization is reported (Pattanayak *et al.*, 2008b; Shukla, 2010; Xing *et al.*, 2009). Rice soils in the IGPs are rich in illites, which fix K^+ both under dry and moist conditions. Malvolta (1983) reported that when soils were shaken with a K solution, K^+ fixation was 25%, while it was 68% when K^+ saturated soils were dried. K^+ fixation was 2–3 times greater after drying than after wetting. Thus, drying cycles in ARS/PARS may create temporary K deficiency in rice.

4.1.7. Sulfur

Sulfur is now recognized as the fourth major nutrient after NPK. Out of the 49,194 soil samples collected all over India under TSI-FAI-IFA project, 46% analyzed low in available S. In all 85 field, experiments were conducted on rice in 12 states of India. In 53 of these experiments, rice yield increase was 25% over check at 30 kg S/ha (Tewatia *et al.*, 2007). Response of rice is reported for both continuously flooded conditions as well as under irrigated conditions (PARS; Gill and Singh, 2009). Sulfur recommendations for rice in India based on 0.15 $CaCl_2$-extractable sulfur (kg/ha) in soils are as follows: 5–10 (low) 45 kg S/ha, 10–15 (medium) 30 kg S/ha, and 15–20 (high) 15 kg S/ha (Singh, 2001). In China also more than 30% of arable soils are deficient in S and S fertilization is recommended in cereals at 20–40 kg/ha (Moris, 2007). In rice–rice cropping system in Guangdon province of China, a negative S balance of 6 kg/ha/yr was obtained when no S was applied, while application of S at 30 kg S/ha changed it to 19.5 kg/ha/yr (Messick and Fan, 2007).

Shifting from continuous flooded conditions to ARS/PARS is likely to increase S availability because aerobic conditions lead to an abundance of $SO_4^{=}$ form, in which S is taken up by crop plants. On the contrary under flooded conditions, $SO_4^{=}$ may be reduced to sulfide leading to the

formation of H_2S, which can lead to sulfide toxicity in rice known as "Akiochi" disease in Japan (Gowarikar *et al.*, 2009).

4.1.8. Iron

Under oxidized conditions, availability of Fe is so low that the roots of plants, especially those belonging to Graminae family, produce phytosiderophores (mugineic acid, etc.; Romheld and Marschner, 1990), which chelate Fe^{2+} and make it available to plants. However, production of phytosiderophores is very low in rice and partly explains its sensitivity to Fe deficiency (Mori *et al.*, 1991). Fe deficiency can spring up in upland rice nurseries (authors' observation) or during preflooding seedling establishment (Synder and Jones, 1991). Flooding the nursery area or fields can overcome such deficiency. Several fold increase in DTPA-extractable Fe under submergence has been reported (Mandal and Das, 2002; Weil and Holah, 1989). However, DTPA-extractable Fe was significantly reduced when soil moisture was maintained at 0.2 or 1 bar (Pal *et al.*, 2008) as compared to that at saturation. Singh *et al.* (2002) reported that DTPA-extractable Fe in 0–15 cm layer was about 5 mg/kg soil in continuously submerged soil as compared to 2.1–2.5 mg/kg soil under different PARS treatments. Rice varieties differ in their tolerance to Fe deficiency (Singh *et al.*, 2003). For example, Pal *et al.* (2008) reported that cultivars CT6510-24-1-2 (V1) and IR71525-19-1-1 (V2) performed better than IR36 (V3) and IR64 (V4) under PARS. Fe^{2+} concentration, which is a better indicator of Fe deficiency or sufficiency (Katyal and Sharma, 1980), in whole rice plants was 68.8, 64.5, 50.7, and 43.3 mg/kg DM in V1, V2, V3, and V4 plants, respectively, when 61 kg ferrous sulfate was applied to soil at sowing. It may be pointed out that for lowland conditions, IR36 has been reported to be tolerant to Fe deficiency (Naidu *et al.*, 1981). Thus, ranking of rice cultivars to Fe deficiency sensitiveness may not be the same under ARS/PARS as under continuous flooding.

Recently, there has been considerable interest in biofortification of cereals including rice in Fe and Zn (Graham *et al.*, 2001). In addition to screening of rice varieties for their efficiency in absorption of Fe, genetic engineering tools are also being employed for improving the uptake of Fe from the soil and increasing absorption and storage of Fe (Takahashi *et al.*, 2001). GM rice has been developed that produces both beta-carotene and ferritin. Seven genes have been introduced four for beta-carotene synthesis and three that allow rice kernels to accumulate available Fe through ferritin (Potrykus *et al.*, 1996). Such rice varieties will be very useful for ARS/PARS.

4.1.9. Zinc

Zinc deficient soils and zinc deficiency in rice is reported worldwide (Alloway, 2004; Cakmak, 2009; Fageria *et al.*, 2002; Gao *et al.*, 2006; Norman *et al.*, 2003; Silanpaa, 1990; Yoshida *et al.*, 1973). Zinc deficiency

in rice was first reported by Nene (1966) from GB Pant University of Agriculture and Technology, India and is characterized by the appearance of dark brown spots on the leaves, which in severe deficiency may coalesce to give dark brown color to the entire plant. This deficiency was given the name *khaira* disease due to the dark brown color of the extract of *Acacia catechu*. In India, about 49% soils are deficient in Zn (Behera *et al*., 2009a,b). Reviews on Zn deficiency and response of rice to Zn are available (Prasad, 2006; Rattan *et al*., 1997). Rice cultivars are also known to differ in their sensitivity to Zn deficiency (Giordano and Mortvedt, 1974; Hacisalihoglu and Kochian, 2003; Norman *et al*., 2003; Quijano-Guerta *et al*., 2002; Rengel, 2001; Sakal *et al*., 1989; Singh *et al*., 1981; Slaton *et al*., 2005; Vissuwa *et al*., 2006).

Oxidation of Fe by oxygen released by rice roots causes a reduction of the rhizosphere pH and limits release of Zn from highly insoluble fractions (Kirk and Bajita, 1995). Zn may also be adsorbed onto the surface of iron oxides formed (Gao *et al*., 2002). Under AWD, reduction in soil moisture may restrict the transport of Zn to rice roots. Gao *et al*. (2006) reported from Beijing that Zn concentration in rice plants at tillering as well as physiological maturity stages was lower under ARS than under flooded conditions. His studies included a japonica lowland rice cultivar Qiuguang and five anaerobic rice cultivars HD72, HD277, HD297, 89B, and K150. K150 was found to be Zn deficient genotype, while 89B and HD277 were more Zn efficient and showed higher Zn concentration at tillering under ARS and flooded conditions.

4.1.10. Other plant nutrients

In recent years, Mg deficiency in rice-based cropping systems and response to Mg has been reported in India (Gill and Singh, 2009). Mg nutrition of rice is important in acid soils, especially under ARS/PARS, because it is required to mediate Al-toxicity in rhizosphere (Yang *et al*., 2007).

Mn deficiency is reported in wheat grown after rice on sandy loam soils of Punjab state of India (Takkar and Nayyar, 1981) and on calcareous purple soils in the upper Sichuan province of China (Hu *et al*., 1981). However, on acid red and lateritic soils Mn could reach toxicity levels, both for rice and wheat (Prasad, 2005).

In India 33% of soils are deficient in B, the deficiency being most (49–69%) in the rice growing states of Orissa, Tamil Nadu, Andhra Pradesh, West Bengal, and Uttar Pradesh (eastern) (Behera *et al*., 2009a,b). In 34 out of 49 on-farm trials in the eastern states of India, an increase of 200 kg/ha was obtained due to B fertilization (Shrotriya and Phillips, 2002). To meet B requirement of crops in eastern India, a boronated-NPK fertilizer (10-26-26-0.3B) has been developed and in 11 on-farm trials on red and lateritic soils of West Bengal and Jharkhand states, an increase in rice yield of 3–20% over NPK was recorded (Sarkar *et al*., 2006).

Mo deficiency in wheat grown after rice in acid soils of the Yangtze river plain in china was reported by Shihua and Wanqiang (2000).

4.1.11. Multinutrient deficiencies

While the above discussion has been on individual plant nutrients, multi-nutrient deficiencies are emerging in many rice growing areas and research to identify has been initiated in India (Behera *et al.*, 2009a,b; Khurrana *et al.*, 2008; Singh *et al.*, 2008). In the Philippines precision, agriculture for small-scale rice farmers has been developed by IRRI and a Nutrient Manager decision tool for rice has been released and is being used with CD and website applications (Buresh, 2010).

4.2. Biotic stresses

Review here is restricted to weeds and soil-borne pathogens.

4.2.1. Weeds

Weeds are the most yield-limiting constraint in aerobic rice production, responsible for about 50% in yield gap (Balasubramanian and Hill, 2002; WARDA, 1996), Hand weeding is the most common practice and leads to drudgery to women and children in the Asian farm families, who do most weeding. Generally two to three weeding are required in ARS needing about 190 man-days/ha (Roder, 2001). Also some weeds such as *Echinochloa* sp. and *Ischaemum* sp. mimic rice during early growth stages (Leeper, 2010) and make hand weeding complicated. Herbicide recommendations are available for different systems of growing rice (Das, 2008), but poor Asian farmers can ill afford the expensive herbicides. Further intensive herbicide applications can lead to environmental contamination and development of herbicide resistance (Carey *et al.*, 1995; Fischer *et al.*, 1993; Lemerle *et al.*, 2001). Efforts are therefore underway to develop weed competitive rice cultivars (Fischer *et al.*, 2001; Pester *et al.*, 1999).

Studies on weed competitiveness of rice cultivars can be made in plots, where weeds are allowed to grow (direct method), but screening a large number of cultivars, year after year, can lead to multiplication of weeds on a farm and is not desirable. Falconer (1989) suggested that traits measured under weedy and weed-free conditions can be thought of as correlated traits expressed by a single genotype in separate environments. Atlin *et al.* (2001) observed that correlated response under weed competition to selection under weed-free conditions is a function of their hereditability of the selection criterion under weed-free conditions, its genetic correlation with the target traits under weed competition and selection intensity. Screening of rice cultivars for weed competitiveness under weed-free conditions using appropriate traits is a more practical indirect method. A three-year study at the IRRI, Philippines, with 40 aerobic and upland rice cultivars showed that

vegetative vigor 2 weeks after sowing was the best trait for selection for their weed competitiveness, the other traits studied were plant height, productive tillers, days to flower, spikelets per panicle, filled grain percentage, grain yield, and harvest index (Zhao *et al*., 2006). The vegetative vigor at 2 weeks after seeding explained 87% of cultivar variation in weedy yield and 40% variation in weed biomass. In this study, cultivars BR144F-MR-6-0-0 and Way Rarem having an early vigor score of 7.2 and 7.1 (on 1–9 scale) had the lowest weed biomass of 128.2 and 128.9 g/m^2, respectively, and gave good yields of 3.28 and 3.04 t/ha, respectively. African rice species *Oryza glaberrima* is noted for its early vigor and high specific leaf area (SLA) and interspecific crosses were made to have high early vigor and SLA (Dingkuhn *et al*., 1999). The newly developed interspecific crosses had thin and lax leaves early in the rice growth period, but as the plants grew older their leaves became thick and erect, permitting better light penetration leading to better production (Table 6).

4.2.2. Soil-borne pathogens

Evidence for soil-borne pathogenic fungus *Gaemannomyceas graminis* var. *graminis* in upland rice was reported from Brazil (Prabhu and Filippi, 2002). Similarly, *Pythium aristsporum* and other saprophytic fungi were considered the cause of failure in rice nursery, when the soil for the nursery was obtained from paddy fields (Furuya *et al*., 2003, 2005).

The most damaging soil-borne pathogen for aerobic rice is root-knot nematode (RKN) *Meloidogyne graminicola* Goldem & Birch (MG) (Arayarungsarit, 1987; Nishizawa *et al*., 1971; Padgham *et al*., 2004; Soriano and Reversat, 2003). MG was first reported in 1963 from the Louisiana State University, Baton Rouge, USA (McGowan and Langdon, 1989). MG is incapable of entering the rice roots under flooded conditions,

Table 6 Performance of some conventional upland and recently developed aerobic rice varieties at IRRI under sprinkler irrigation

Cultivar	Grain (t/ha)	Height (cm)	Lodging (%)	Days to 50% flowering (d)
Magat	4.27	80	0	83
IR55423-01	3.54	111	0	80
Maravilha	3.04	113	1	72
KMP34	2.98	81	0	77
B6144	2.50	116	96	75
LSD (0.05)	0.72	6	–	3

Note: Magat and IR55423-01 are aerobic rice varieties (ARVs) developed at IRRI, Philippines, while Maravilha is an ARV developed at CNPAF, Brazil, and B6144 is a conventional rainfed variety.
From George *et al*. (2002).

Table 7 Grain yield and root-knot nematode (RKN) galling at flowering in the roots of aerobic rice Apo at Tarlac, Philippines

Treatment	Grain (t/ha)		RKN*	
	2006	2007	2006	2007
Direct seeded rice (DSR)	0.2b	0.0b	3.7a	4.8a
Biocide + DSR	2.2a	2.4a	0.3b	2.4b
Transplanted rice (TPR)	0.3b	0.0b	4.4a	4.9a

Note: 1. Value followed by same suffix do not differ significantly (p=0.05).
2. RKN—degree of galling on a scale of 1–5.
3. Biocide-Dazomet at 50 g a.i./m^2, 6–7 weeks before seeding.
Adapted from Kreye *et al.* (2009b).

although it can survive for extended periods under such conditions (Bridge and Page, 1982) and attacks rice roots when aerobic conditions come up. In a study in Philippines, RKNs were found to be most damaging pathogen for aerobic rice Apo (Kreye *et al.*, 2009b) (Table 7). In untreated plots, the rice yield was 0.2–0.3 t/ha in 2006 and nil in 2007. However, in plots treated with nematicide Dazomet yield of 2.2 t/ha was obtained in 2006 and 2.4 t/ha in 2007. In the first year, degree of galling in rice roots was only 0.4 in the nematicide-treated plots, while it was 3.4–4.4 in untreated plots. In 2007, galling increased even in nematicide-treated plots to 2.4, while it was 4.8–4.9 in untreated plots. Heating soil at 120 °C for 4 h is also reported to control soil pathogens (Nie *et al.*, 2007). For poor Asian farmers, natural plant derived biocides, such as, those from Neem (*Azadirachta indica* Juss) may be cheaper, indigenously available and ecofriendly. Also pathogens cannot easily develop resistance against neem products because they have more than one molecule responsible for biocidal activity. Neem products have been reported to have fungicidal, insecticidal and nematicidal, and antiviral properties (Prasad *et al.*, 2007).

5. Making ARS/PARS Sustainable

Monoculture of a cereal is not sustainable (Power and Follet, 1987) and it is necessary that a crop rotation preferably involving a legume is practiced. The soil fertility restoring effect of legumes such as beans (*Vicia faba*), various clovers, *medicago* sp. lupins (*Lupinus album*), and vetch (*Vicia sativa*) is well known (Bullock, 1992; Prasad and Power, 1997). Introduction of a summer green manure or a dual purpose legume has been suggested as a way to make rice–wheat (*Triticum aestivum*), a cereal–cereal rotation in the IGPs more sustainable and less dependent on fertilizer

Table 8 Yield of rice aerobic rice Carajas after 5 years of monocropping or after 4 and 2 years of rice followed by soybean

Cropping system	Rice yield (t/ha)
Rice monoculture (5 years)	1.16
Rice (4 years)–soybean (1 year)	2.58
Rice (2 years)–soybean (3 years)	4.32

Adapted from Pinheiro *et al.* (2006).

nitrogen (Prasad, 2005; Sharma and Prasad, 1999; Sharma *et al.*, 2000). In a five-year study with ARS in Brazil, rice monocropping gave an yield of 1.16 t/ha as compared to 2.58 t/ha after 1 year of soybean (Glycine max) and 4.32 t/ha after 5 years of soybean (Guimaraes *et al.*, 2001a; Pinheiro *et al.*, 2006) (Table 8).

As regards nutrient deficiencies, slow release N fertilizers and nitrification can well fit in ARS/PARS, where N losses are likely to be high (Kanneta *et al.*, 1994; Prasad and Power, 1995; Prasad *et al.*, 1971). On acid soils, which have high P-fixation capacity (Sanchez and Uehara, 1980) ground rock phosphate could be a better choice for meeting P requirement of rice (Mathur *et al.*, 1979; Rajan *et al.*, 1996), especially in association with phosphate solubilizing bacteria (Sharma *et al.*, 2010). Zinc deficiency can be easily overcome by soil application of zinc fertilizers or zinc-coated urea (Shivay *et al.*, 2008a,b), foliar application of Zn fertilizers (Slaton *et al.*, 2005; Dhaliwal *et al.*, 2010) or coating of rice with zinc (Slaton *et al.*, 2001). As regards Fe, foliar application is the best way (Singh *et al.*, 2003). These are the leads from experiments conducted on lowland flooded rice, but such studies are needed for ARS to find out the applicability or otherwise of these techniques.

6. Conclusion and Future Research Thrust Areas

Keeping in view the scarcity of irrigation water for rice, ARSs have been developed for cool temperate conditions of northern China and Brazil. How far these systems can be extended to warm tropical and subtropical regions is yet to be verified. Subtropical regions, where most irrigated rice is grown, have fairly high temperatures and ET loss, the plausible solution is PARSs. A wide variety of choices of technologies for saving water in rice production and practicing PARS exist. However, these could not be extended to large areas because of the government policy in some regions of giving electricity at reduced or no cost to the farmers for boosting rice

production. This has resulted in overuse of irrigation water at the cost of irreparable depletion of the groundwater which is a matter of great concern. PARS can be extended even to upland rice regions with the help of microirrigation, where one or two life saving irrigations can almost double or treble the rice yield. Such an effort will help the poorest of the poor rice farmers of the world.

On the research front much needs to be done on the nutrient dynamics in soils under ARS and PARS. Also research is needed on soil ecology in rice soils. There is good diversity in rice genomes and with the availability of new modern tools in genetic engineering, it may be possible to develop in future rice genotypes that can grow under aerobic environment as other cereals such as corn, wheat, barley, etc.

REFERENCES

Aggarwal, G. C., Sidh, A. S., and Singh, N. T. (1987). Effect of periods of moisture stress at different fertility levels on rice. *J. Indian Soc. Soil Sci.* **35,** 280–283.

Aggarwal, M. K., Panda, S. N., and Panigrihi, B. (2004). Modelling water balance parameters for rainfed rice. *J. Irrig. Drain. Eng.* **130,** 129–139.

Ali, M. T., and Shahram, S. H. (2007). Converter slag as a liming agent in the amelioration of acidic soils. *Int. J. Agric. Biol.* **9,** 715–720.

Ali, M. L., Pathan, M. S., Zhang, J., Bai, G., Sarkarung, S., and Nguyen, H. T. (2000). Mapping QTLs for root traits in a recombinant inbred population from two indica ecotypes in rice. *Theor. Appl. Genet.* **101,** 756–766.

Alloway, B. J. (2004). Zinc in Soils and Crop Nutrition. International Zinc Association, Brussels, Belgium.

Angus, J. F., Hasegowa, S., Hsiao, T. C., Liboon, S. P., and Zandstra, H. G. (1983). The water balance of post-monsoonal dryland crops. *J. Agric. Sci. Camb.* **101,** 699–710.

Arayarungsarit, L. (1987). Yield ability of rice in fields infested with root-knot nematode. *Int. Rice Res. Newsl.* **12,** 14.

Atlin, G. N., Cooper, M., and Bjornstad, A. (2001). A comparison of formal and participatory breeding approaches using selection theory. *Euphytica* **122,** 463–475.

Atlin, G. N., Lafitte, H. R., Tao, D., Laza, M., Amanle, M., and Courtois, B. (2006). Developing rice cultivars for high fertilizer upland systems in Asian tropics. *Field Crops Res.* **97,** 43–52.

Aulakh, M. S., Khera, M. S., Doran, J. W., and Bronson, K. F. (2001). Denitrification, nitrous oxide and carbon dioxide fluxes in rice–wheat cropping system as affected by crop residues, fertilizer nitrogen and green manure. *Biol. Fertil. Soils* **34,** 375–389.

Balasubramanian, V., and Hill, J. E. (2002). Direct seeding of rice in Asia: Emerging issues and strategic research needs for the 21st century. *In* "Direct Seeding: Research Strategies and Opportunities" (S. Pandey, *et al.*, Eds.), pp. 15–39. IRRI, Los Banos, Philippines.

Banoc, D. M., Yamauch, A., Kamoshita, A., Wade, L. J., and Pardales, J. R. (2000). Dry matter production and root-system development of rice cultivars under fluctuating moisture. *Plant Prod. Sci.* **3,** 197–207.

Barker, R., Dave, D., Tuong, T. P., Bhuuiyan, S. I., and Guerra, L. C. (1999). The outlook for resources in the year 2020: Challenges for research on water management in rice production. *In* "Assessment and Orientation Towards 21st Century. Proceedings of 19th

Session, International Rice Commission, Cairo, Egypt, 7–9 September 1998, FAO, Rome," pp. 96–109.

Barrett, S. C. H., and Seaman, D. E. (1980). The weed flora of California rice fields. *Aquat. Bot.* **9,** 351–376.

Batchelor, C. H., and Roberts, J. (1983). Evaporation from the irrigation water, foliage and panicles of paddy rice in north east of Sri Lanka. *Agric. Meteorol.* **29,** 11–20.

Beecher, H. G., Dunn, B. W., Thompson, J. A., Humphreys, E. S., Mathews, K., and Timsina, J. (2006). Effect of raised beds, irrigation and nitrogen management on growth, water use and yield of rice in south-eastern Australia. *Aust. J. Exp. Agric.* **40,** 1363–1372.

Behera, S. K., Singh, D., and Dwivedi, B. S. (2009a). Change in fractions of iron, manganese, copper and zinc in soil under continuous cropping for more than three decades. *Commun. Soil Sci. Plant Anal.* **40,** 1–28.

Behera, S. K., Singh, M. V., and Lakaria, B. L. (2009b). Micronutrient deficiencies in Indian soils and their amelioration through fertilization. *Indian Farming* **59**(2), 28–31.

Belder, P., Bouman, B. A. M., Cabangon, R., Lu, G., Quilang, E. J. P., Li, Y., Spiertz, J. H. J., and Tuong, T. P. (2004). Effect of water saving irrigation on rice yield and water use in typical lowland conditions in Asia. *Agric. Water Manage.* **65,** 193–210.

Belder, P., Bouman, B. A. M., Spiertz, J. H. J., Peng, S., Castaneda, A. R., and Visperas, R. M. (2005). Crop performance, nitrogen and water use in flooded and aerobic rice. *Plant Soil* **273,** 167–182.

Bhagat, R. M., Bhuiyan, S. I., Moody, K., and Estorninos, L. E. (1999). Effect of water, tillage and herbicides on ecology of weed communities in intensive water seeded rice ecosystem. *Crop Protect.* **18,** 293–303.

Bhat, J. A., Mandal, B., and Hazra, G. C. (2007). Basic slag as a liming material to ameliorate soil acidity in Alfisols of sub-tropical India. *Am. Eurasian J. Agric. Environ. Sci.* **2,** 321–327.

Bhat, J. A., Kundu, M. C., Hazra, G. C., Santra, G. H., and Mandal, B. (2010). Rehabilitating acid soils for increasing crop productivity through low-cost liming materials. *Sci. Total Environ.*10.1016/j.scitotenv.2010.07.011.

Blum, A. (1988). Plant Breeding for Stress Environments. CRC Press, Boca Raton, FL.

Bonilla, P., Dvorak, J., Mackill, D. J., Dcal, K., and Gregario, G. (2002). RFLP and SSLP mapping of salinity tolerance genes in chromosome 1 of rice (*Oryza sativa* L.) using recombinant inbred lines. *Philipp. Agric. Scientist.* **85,** 68–76.

Boonjung, H., and Fukai, S. (1996). Effects of water deficit at different growth stages on rice growth and yield under upland conditions. 2. Phenology, biomass production and yield. *Field Crops Res.* **48,** 47–55.

Borell, A., Garside, A., and Fulai, S. (1997). Improving efficiency of water for irrigated rice in a semi-arid environment. *Field Crops Res.* **52,** 231–248.

Bouman, B. A. M. (2001). Water efficient management strategies in rice production. *Int. Rice Res. Notes* **16**(20), 17–22.

Bouman, B. A. M., and Tuong, T. P. (2001). Field water management to save water and increase its productivity in irrigate rice. *Agric. Water Manage.* **49,** 11–30.

Bouman, B. A. M., and van Laer, H. H. (2006). Description and evaluation of the rice growth model ORYZA2000 under nitrogen limited conditions. *Agric. Syst.* **87,** 249–273.

Bouman, B. A. M., Xiaoguang, Y., Huaqi, Y., Zhiming, W., Junfang, Z., Changui, W., and Bin, C. (2002). Aerobic rice (Han Dao): A new way of growing rice in water-short areas. *In* "Proceedings of the 12th International Soil Conservation Conference, Beijing, 26–31 May 2002," pp. 175–181. Tsinghua University Press, Beijing.

Bouman, B. A. M., Peng, S., Castaneda, A. R., and Visperas, R. M. (2005). Yield and water use of irrigated tropical aerobic rice systems. *Agric. Water Manage.* **74,** 87–105.

Bouman, B. A. M., Humphreys, E., Truog, T. P., and Barker, R. (2007). Rice and water. *Adv. Agron.* **92,** 187–237.

Bridge, J., and Page, S. L. G. (1982). The rice root-knot nematode *Meloidogyne graminicola* on deep water rice (*Oryza sativa* subsp. Indica). *Rev. Nematol.* **5,** 225–232.
Bucks, D. A., Nakayama, F. S., and Warrik, A. W. (1982). Principles, practices and potentialities of trickle irrigation. *Adv. Irrig.* **1,** 220–291.
Bullock, D. G. (1992). Crop rotation. *Crit. Rev. Plant Sci.* **11,** 309–326.
Buresh, R. J. (2010). Precision agriculture for small-scale farmers. *Rice Today* **9**(3), 46.
Cakmak, I. (2009). Enrichment of fertilizers with zinc—An excellent investment for humanity and crop production in India. *J. Trace Elem. Med. Biol.* **23,** 981–989.
Carey, V. F., Hoagland, R. E., and Talbert, R. E. (1995). Verification and distribution of propanil resistant barnyard grass (*Echinochloa crusgalli*) in Arkansa. *Weed Technol.* **9,** 366–372.
Carriger, S., and Vallee, D. (2007). More crop per drop. *Rice Today* **6**(2), 10–13.
Castaneda, A. R., Bouman, B. A. M., Peng, S., and Visperas, R. M. (2003). The potential of aerobic rice to reduce water use in water-scarce irrigated low-lands in the tropics. *In* "Water-Wise Rice Production" (B. A. M. Bouman, H. Hengsdijk, B. Hardy, P. S. Bindraban, T. P. Tuong, and J. K. Ladha, Eds.), International Rice Research Institute, Los Banos, Philippines.
Chaudhary, T. N. (1997). Water Requirement of Rice for Efficient Production. Directorate of Water Management Research, Patna, India.
Clark, L. J., Aphale, S. L., and Barraclough, P. B. (2000). Screening the ability of rice roots to overcome the mechanical impedance of wax layers: Importance of test conditions and measurement criteria. *Plant Soil* **219,** 187–196.
Clough, T. J., Sherlock, R. R., and Kelliher, F. M. (2003). Can liming mitigate N_2O fluxes from a urine amended soil? *Aust. J. Soil Res.* **41,** 439–457.
Connor, D. J., Timsina, J., and Humphreys, E. (2002). Prospects fpr permanent beds in the rice-wheat system. *In* "Improving the Productivity and Sustainability of Rice-Wheat Systems: Issues and impacts" (J. K. ladha, J. E. Hill, J. M. Duxbury, R. K. Gupta, and R. J. Buresh, Eds.), Vol. 65, pp. 197–210. ASA Special Publication, ASA Inc., CSA Inc., SSSA Inc., Madison, WI.
Courtois, B., and Lafitte, R. (1999). Improving rice for drought prone upland environments. *In* "Genetic Improvement of Rice for Water-Limited Environments" (O. Ito, J. O. Toole, and B. Hardy, Eds.), pp. 35–56. International Rice Research Institute, Los Banos, Philippines.
Cruz, R. T., and O'Toole, J. C. (1984). Dryland rice response to an irrigation gradient at flowering stage. *Agron. J.* **76,** 178–183.
Das, T. K. (2008). Weed Science-Basics and Applications. Jain Brothers, New Delhi, 901 pp.
Dastane, N. G. (1967). A Practical Manual for Water Use Research. Navbharat Prakashan, Pune, India.
Dastane, N. G., and Nelliat, E. V. (1970). Effect of Different Water Regimes on Rice.Ann. Rept. Division of Agronomy, Indian Agricultural Research Institute, New Delhi, India.
De Datta, S. K., Fuye, F. G., and Mallick, R. N. (1975). Soil water relations in rice. *In* "Proceedings of the Seminar on. Soil Management in Tropical America" (E. Bornamisze and A. Alvarado, Eds.), pp. 168–185. CIAT, USAID, Cali, Columbia.
Dhaliwal, S. S., Sadana, U. S., Khurrana, M. P. S., Dhadli, H. S., and Manchanda, J. S. (2010). Enrichment of rice grains with zinc and iron through ferti-fortification. *Indian J. Fert.* **6**(7), 28–39.
Dhillon, S. S., Hobbs, P. R., and Samra, J. S. (2000). Investigations on bed planting systems as an alternative tillage and crop establishment practice for improving wheat yields sustainably. *In* "Proceedings of the 15th Conference International Soil Tillage Research Organisation, 2–7 July 2000, Fort Worth, Texas".
Dingkuhn, M., Johnson, D. E., Sow, A., and Audebert, A. Y. (1999). Relationship between upland rice canopy characteristics and weed competitiveness. *Field Crops Res.* **61,** 79–95.

Dobermann, A., Cassman, K. G., Mamaril, C. P., and Sheeley, J. E. (1998). Management of phosphorus, potassium and sulfur in intensive irrigated wetland rice. *Field Crops Res.* **56,** 113–138.

Dunn, D., and Stevens, G. (2007). Phosphorus management in a dry-seeded, delayed, flooded rice production system (North America). *Better Crops* **91**(1), 20–21.

Ekanayke, I. J., De Datta, S. K., and Steponkus, P. L. (1989). Spikelet sterility and flowering response of rice to water stress at anthesis. *Ann. Bot.* **63,** 257–264.

Fageria, N. K. (2000). Upland rice response to soil acidity in Cerrado soil. *Pesqui. Agropecu. Bras.* **35,** 2303–2307.

Fageria, N. K., and Baligar, V. C. (2003). Upland rice and allelopathy. *Commun. Soil Sci. Plant Anal.* **34,** 1313–1329.

Fageria, N. K., and Baligar, V. C. (2008). Ameliorating soil acidity of tropical oxisols by liming for sustainable crop production. *Adv. Agron.* **99,** 345–399.

Fageria, N. K., Baligar, V. C., and Clark, R. B. (2002). Micronutrients in crop production. *Adv. Agron.* **77,** 185–268.

Fageria, N. K., Castro, E. M., and Baligar, V. C. (2004). Response of upland rice genotypes to soil acidity. *In* "The Red Soils of China: Their Nature, Management and Utilization" (M. J. Wilson, Z. He, and X. Yang, Eds.), pp. 219–237. Kluwer Academic Publishers, Dordrecht, Netherlands.

FAI (2009). Fertilizer Statistics 2009–09. The Fertilizer Association of India, New Delhi.

Falconer, D. S. (1989). Introduction to Quantitative Genetica. 3rd edn. Longmans, London.

Farooq, M., Kobayashi, N., Wahid, A., Ito, O., and Basar, S. M. A. (2009). Strategies for producing more rice with less water. *Adv. Agron.* **101,** 351–388.

Feng, L., Bouman, B. A. M., Tuong, T. P., Cabangon, R. J., Yanglang, Li, Guoan, L., and Feng, Y. (2007). Exploring options to grow rice using less water in northern China. I. Field experiments and model evaluation. *Agric. Water Manage.* **88,** 1–13.

Fischer, A. J., Granados, E., and Trujillo, D. (1993). Propanil resistance in populations of jungle rice (*Echinochloa colona*) in Colombian rice fields. *Weed Sci.* **41,** 201–206.

Fischer, A. J., Ramirez, H. V., Gibson, K. D., and Pinheiro, B. D. S. (2001). Competitiveness of semi-dwarf upland rice cultivars against palisade grass (*Brachiaria brizantha*) and signal grass (*B. decunmbens*). *Agron. J.* **93,** 967–973.

Flowers, T. J., and Yeo, A. R. (1981). Variability in resistance to sodium chloride salinity within rice (*Oryza sativa* L.) varieties. *New Phytol.* **88,** 363–373.

Foy, C. D. (1992). Soil chemical factors limiting plant growth. *Adv. Soil Sci.* **19,** 97–149.

Fukai, S., and Cooper, M. (1995). Review: Development of drought-resistant cultivars under physio-morphological traits in rice. *Field Crops Res.* **40,** 67–86.

Furuya, H., Matsumoto, T., Fuji, S., and Naito, H. (2003). Inconspicuous resistant of rice seedling growth by root-infecting fungi in soil of a rice paddy field. *J. Gen. Plant Pathol.* **69,** 115–119.

Furuya, H., Tubaki, K., Matsumoto, T., Fuji, S., and Naito, H. (2005). Deleterious effects of fungi from paddy soils on seminal root of rice. *J. Gen. Plant Pathol.* **71,** 333–339.

Gao, S., Tanji, K. K., Scardaci, S. C., and Chow, A. T. (2002). Comparison of redox indicators in a paddy soil during rice growing season. *Soil Sci. Soc. Am. J.* **66,** 805–817.

Gao, X., Zou, C., Fan, X., Zhang, F., and Hoffland, E. (2006). From flooded to aerobic conditions in rice cultivation: Consequences from Zn uptake. *Plant Soil* **280,** 41–47.

Gautam, R. K., Singh, R. K., Mishra, B., Qadar, A., Singh, G., Singh, Y. P., Sharma, S. K., and Ajore, R. (2009). Basmati CSR30(Yamini)—The First Salt Tolerant Basmat Variety is a Boon to the Farmers. Central Soil Salinity Research Institute, Karnal, IndiaTech Bull.2009/6, 10 pp.

George, T., Magabanua, R., Garrity, D. P., Tubana, B. S., and Quiton, J. (2002). Rapid yield loss of rice cropped successively in aerobic soil. *Agron. J.* **84,** 981–989.

Gill, M. S., and Singh, V. K. (2009). Productivity enhancement of cereals through secondary and micronutrients application. *Indian J. Fert.* **5**(4), 59–106.

Giordano, P. M., and Mortvedt, J. J. (1974). Response of several rice cultivars to Zn. *Agron. J.* **66,** 220–223.

Gleik, P. R., (Ed.) (1993). *In* "Water Crisis: A Guide to the World's Fresh Water Resources. Pacific Institute for Studies in Drought Environment and Security", p. 473. Stockholm Environment Institute, Oxford University Press, New York.

Goff, S. A., Ricke, D., Lau, T. H., Presting, G., Wang, R., Dunn, M., Glazebrook, J., Sessions, A., Oeller, P., Verma, H., *et al.* (2002). A draft sequence of the rice genome (*Oryza sativa L.* ssp. *Japonica*). *Science* **296,** 92–100.

Gowarikar, V., Krishnamurthy, V. N., Gowarikar, S., Dhanokar, M., and Paranjape, K. (2009). The Fertilizer Encyclopaedia. John Wiley, New York.

Graham, R. D., Welch, R. M., and Bouis, H. E. (2001). Addressing micronutrient malnutrition through enriching the nutrient quality of staple foods: Principles, perspectives and knowledge gap. *Adv. Agron.* **70,** 77–142.

Gregorio, G. B., Sanadhira, D., Mendoza, R. D., Manigbas, N. L., Roxas, J. P., and Guerta, C. Q. (2002). Progress in breeding for salinity tolerance and related aerobic stress in rice. *Field Crops Res.* **76,** 96–101.

Grigg, B. C., Beyrouty, C. A., Norman, R. J., Gbur, E. E., Hanson, M. G., and Wells, B. R. (2000). Rice responses to changes in floodwater and N timing in southern USA. *Field Crops Res.* **66,** 73–79.

Grobecker, A. J., Coroniti, S. C., and Cannon, R. H. Jr. (1975). The Effects of Stratospheric Pollution by Aircraft.Report of the Findings of the Climatic Impact Assessment Program (DOT-TST-75-50), Washington, DC, US Dept. of Transportation.

Guerra, L. C., Bhuiyan, S. L., Tuong, T. P., and Barker, R. (1998). Producing More Rice with Less Water from Irrigated Systems. SWIM Paper 5 International Water Management Institute/International Rice Research Institute, Colombo, Sri Lanka.

Guimaraes, C. M., Prabhu, A. S., Castro, E da M. de., Freira, E., Yokoyama, L. P. and Cobucci, T. (2001a). Cultivo do arroz em rotacap com soja. Circular Tecnica 41, Embrapa Arroz e Feijao, Santo Antonio de Goias, Brasil.

Guimaraes, E. P., Viera, N. R. A., and Pinheiro, B. da S. (2001b). Breeding for specialty rice in Latin America: status and perspectives. *In* "Specialty Rices of the World: Breeding, Production and Marketing" (R. C. Chaudhary and V. D. Tran, Eds.), pp. 317–322. FAO, Rome.

Gupta, R. K., Naresh, R. K., Hobbs, P. R., and Ladha, J. K. (2002). Adopting conservative agriculture in the rice–wheat system of the Indo-Gangetic Plain: New opportunities for saving water. *In* "Water-Wise Rice Production" (B. A. M. Bouman, H. Hengsdijk, B. Hardy, P. S. Bindraban, T. P. Tuong, and J. K. Ladha, Eds.), pp. 207–222. International Rice Research Institute, Los Banos, Philippines.

Hach, C. V., Chin, D. V., Dien, T. V., and Luat, N. V. (1997). Study on the effect of water depth and herbicides on weeds and grain yield of rice. *Sci. Proc. Vietnam Natl. Inst. Agric. Sci. Technol.* **5,** 20–21.

Hacisalihoglu, G., and Kochian, L. V. (2003). How do some plants tolerate low levels of soil zinc? Mechanisms of zinc efficiency in crop plants. *New Phytol.* **159,** 341–350.

Hittalmani, S. (2009). Hari-Om Ashram Trust Award Citation for the Biennium 2007–08. Citations Indian Council of Agricultural Research Awards, New Delhip. 26.

Hsiao, T. C., O'Toole, J. C., Yambao, E. B., and Tutner, N. C. (1984). Influence of osmotic adjustment on leaf rolling and tissue death in rice (*Oryza sativa* L.). *Plant Physiol.* **75,** 338–341.

Hu, S. N., Hua, W. Q., and Lu, M. (1981). Studies on the effect of Mn fertilizer on wheat yield on a calcareous soil in Wengiang. *Soil* **13,** 100–103.

Huaqi, W., Bouman, B. A. M., Zhao, D., Changgui, W., and Moya, P. F. (2002). Aerobic rice in northern China: Opportunities and challenges. *In* "Water-Wise Rice Production" (B. A. M. Bouman, H. Hengsdijk, B. Hardy, P. S. Bindraban, T. P. Tuong, and J. K. Ladha, Eds.), pp. 143–154. International Rice Research Institute, Los Banos, Philippines.

Humphreys, E., Muirhead, W. A., Melhuish, F. M., White, R. J. G., and Blackwell, J. L. (1989). The growth and nitrogen economy of rice under sprinkler and flood irrigation in southeast Australia. II. Soil moisture and mineral nitrogen transformations. *Irrig. Sci.* **10,** 201–213.

IRRI (1997). Rice Almanac 2nd edn. International Rice Research Institute, Los Banos, Philippines, 181 pp.

IRRI (2006). Bringing Hope, Improving Lives-Strategies. Plan 2007–2015 International Rice Research Institute, Los Banos, Philippines, 61 pp.

Jat, M. L., Chandra, P., Gupta, R., Sharma, S. K., and Gill, M. A. (2006). Laser land levelling—A precursor technology for resource conservation. Rice–Wheat Consortium Tech. Bull. Vol. 7, Project Directorate for Cropping Systems Research, Modipuram and Rice–Wheat Consortium for the Indo-Gangetic Plains, New Delhi, 38 pp.

Jat, M. L., Gathala, M. K., Singh, K. K., Ladha, J. K., Singh, S., Gupta, R. K., Sharma, S. K., Sahrawat, Y. S., and Tetarwal, J. P. (2008). Experiences with the permanent beds in the rice–wheat systems of the western IGP. *In* "Permanent Beds and Rice Residue Management for Rice–Wheat System in IGP" (E. Humphreys and C. H. Roth, Eds.), pp. 98–107. Australian Centre for International Agricultural Research.

Justin, S. H. F., and Armstrong, W. (1991). Evidence for the involvement of ethylene in aerenchyma formation in adventitious roots of rice. *New Phytol.* **118,** 49–62.

Kanneta, Y., Awasaki, H., and Murai, Y. (1994). The non-tillage rice culture by single application in a nursery box with controlled release fertilizers. *Nippon Dojo Hiryogaku Zashi* **65**(4), 233–281 (in Japanese).

Katyal, J. C., and Sharma, B. D. (1980). A new technique of plant analysis to resolve iron chlorosis. *Plant Soil* **130,** 143–156.

Kawata, S., and Ishihara, S. (1959). Study on the root hairs in rice plants. *Proc. Crop Sci. Soc. Jpn.* **29,** 341–348.

Khan, M. H. R., Kabir, S. M., Bhuiyan, M. M. A., Blum, H. P., Old, Y., and Adachi, T. (2008). Reclamation of Badakhal hot spot of acid sulphate soils in relation to rice production by basic slag and aggregate size treatments under modified plain-ridge ditch techniques. *Soil Sci. Plant Nutr.* **54,** 574–586.

Khurrana, H. S., Bijay-Singh, Dobermann, A., Phillips, S. B., Sidhu, A. S., and Yadvinder-Singh (2008). Site-specific nutrient management performance in a rice–wheat cropping system. *Better Crops-India* **1,** 26–28.

Khush, G. S. (2005). Taxonomy, ecology and agronomy of rice cultivation vis-à-vis genetic engineering of rice. *In* "Biosafety of Transgenic Rice" (V. L. Chopra, S. Shantharam, and R. P. Sharma, Eds.), pp. 26–37. National Academy of Agricultural Sciences, New Delhi, India.

Kirk, G. J. D., and Bajita, J. B. (1995). Root induced iron oxidation, pH changes and zinc solubilisation in rhizosphere of lowland rice. *New Phytol.* **131,** 129–137.

Kirk, G. J. D., George, T., Courtois, B., and Sanadhira, D. (1998). Opportunities to improve phosphorus deficiency and soil fertility in rainfed lowland rainfed and upland rice ecosystem. *Field Crops Res.* **56,** 73–92.

Kreye, C., Bouman, B. A. H., Castaneda, A. R., Lampayan, R. M., Faronilo, J. E., Lactaoen, A. T., and Fernandez, L. (2009a). Possible causes of yield failure in tropical aerobic rice. *Field Crop Res.* **111,** 197–206.

Kreye, C., Bouman, B. A. M., Reversat, G., Fernandez, L., Vera Cruz, C., Elazegui, F., Faronilo, J. E., and Llorca, L. (2009b). Biotic and abiotic causes of yield failure in tropical aerobic rice. *Field Crops Res.* **112,** 97–106.

Ku, H. S., Suge, H., Rappaport, L., and Pratt, H. K. (1970). Simulation of rice coleoptile growth by ethylene. *Planta* **90,** 333–339.

Kukal, S. S., Humphreys, E., and Smith, D. J. (2008). Permanent beds for rice–wheat systems in Punjab, India: Crop performance. *In* "Permanent Beds and Rice Residue Management for Rice–Wheat System in IGP" (E. Humphreys and C. H. Roth, Eds.), pp. 9–19. Australian Centre for International Agricultural Research, Canberra.

Kutschera, U., Siebert, C., Masuda, Y., and Sievers, A. (1990). Effects of submergence on development and gravitropism in the coleoptile of *Oryza sativa. Planta* **183,** 112–119.

Lafitte, H. R., and Bennet, J. (2002). Requirements for aerobic rice: Physiological and molecular considerations. *In* "Water-Wise Rice Production" (B. A. M. Bouman, H. Hengsdijk, B. Hardy, P. S. Bindraban, T. P. Tuong, and J. K. Ladha, Eds.), pp. 259–274. International Rice Research Institute, Los Banos, Philippines.

Lafitte, H. R., Courtois, B., and Arraudeau, M. (2002). Genetic improvement of rice in aerobic systems: Progress from yield to genes. *Field Crops Res.* **75,** 171–190.

Lafranco, B. (2010). Pressure in the south. *Rice Today* **9**(3), 17.

Leeper, J. R. (2010). Back to basics in improving yield by controlling weeds. *Rice Today* **9**(3), 12–14.

Lemerle, D., Gill, D. S., Murphy, C. E., Walker, S. R., Cousens, R. D., Mokhtari, S., Peltzer, S. J., Coeman, R., and Luchett, D. J. (2001). Genetic improvement and agronomy for enhanced wheat competitiveness with wheat. *Aust. J. Agric. Res.* **52,** 527–548.

Levitt, J. (1980). Response of Plants to Environmental Stresses. Water, Radiation, Salt and other Stresses. **Vol. II,** Academic Press, New York.

Lilly, J. M., and Ludlow, M. M. (1996). Expression of osmotic adjustment and dehydration tolerance in diverse rice lines. *Field Crops Res.* **48,** 185–197.

Luo, Y. F., Khan, S., Cui, Y. L., Feng, Y. H., and Li, Y. L. (2006). Modeling for water balance for aerobic rice: A dynamics approach. *In* "MODSIM 2005. Proceedings of the International Congress Modeling and Simulation, Melbourne, Australia," pp. 1860–1866.

Maji, A. K., ObiReddy, G. P., and Sarkar, D. (2010). *In* "Degraded and Wastelands of India-Status and Spatial Distribution" (S. M. Virmani, R. Prasad, and P. S. Pathak, Eds.), p. 158. National Academy of Agricultural Sciences, New Delhi, India.

Malvolta, E. (1983). Potassium status of tropical and sub-tropical soils. *In* "Potassium in Agriculture" (R. D. Munson, Ed.), pp. 163–200. Am. Soc. Agron. Madison, WI, USA.

Mandal, L. N. (1979). Transformation of phosphorus in water-logged soil. *Bull. Indian Soc. Soil Sci.* **12,** 73–80.

Mandal, S. K., and Das, B. (2002). Availability and mobilization of iron and manganese in some soils of West Bengal. *Indian Agric.* **46,** 209–218.

Martin, G. J., Padmanathan, P. K., and Subramanian, E. (2007). Identification of suitable rice variety adaptability to aerobic irrigation. *J. Agric. Biol. Sci.* **2**(2), 1–3.

Mathur, B. S., Jha, K. K., Lal, S., and Srivastava, B. P. (1979). Utilization of phosphate rock deposits in rice soils of Chotanagpur. *Bull. Indian Soc. Soil Sci.* **12,** 505–572.

McGowan, J. B., and Langdon, R. K. (1989). Hosts of the rice root-knot nematode Meloidogyne graminicola. Florida Dept. Agric. & Consumer Service, Dept. of Industry, Nem. Cicular 172.4 pp.

Mckill, D. J., Coffman, W. R., and Garrity, D. P. (1996). Rainfed Lowland Rice Improvement. IRRI, Los Banos, Philippines, 242 pp.

Mehlhorn, H. (1992). Ethylene promoted ascorbate peroxidase activity protects plants against hydrogen peroxide, ozone and paraquat. *Plant Cell Environ.* **13,** 971–976.

Meisner, C. A., Acevedo, E., Flores, D., Sayre, K., Ortiz-Monasterio, I., Byrlee, D., and Limon, A. (1992). Wheat production and grower practices in the Yaqui valley, Sonora, Mexico.Wheat Special Report 6 CIMMYT, Mexico.

Mendoza, T. L. (2010). AWD goes top Africa. *Ripple* **5**(2), 7.

Menegus, F., Cattaruzza, L., Scaglioni, L., and Ragg, E. (1992). Effects of oxygen levels on the metabolism and development of seedlings of *Trapa natans* and two ecologically related species. *Physiol. Plant.* **86,** 168–172.

Messick, D. L., and Fan, M. X. (2007). Sulfur in balanced fertilization in China. *In* "Proceedings of the FAI-IFA Workshop on Sulfur in Balanced Fertilization" (M. C. Sarkar, B. C. Biswas, and S. Dev, Eds.), pp. 1–12. Fertilizer Association of India, New Delhi.

Mohapatra, S. (2010). Pockets of gold. *Rice Today* **9**(2), 32–33.

Moore, P. A. Jr., Attananda, T., and Patrick, W. H. Jr. (1990). Factors affecting rice growth on acid sulphate soils. *Soil Sci. Soc. Am. J.* **54,** 1651–1656.

Mori, S., Nishizawa, N., Hyashi, H., Chino, M., Yoshimura, E., and Ishihara, I. (1991). Why are young rice plants highly susceptible to iron deficiency. *Plant Soil* **130,** 143–156.

Moris, R. J. (2007). Sulfur in agriculture: International perspective. *In* "TSI-FAI-IFA Sulfur Project-Salient Findings. Proceedings of the TSI-FAI-IFA Symposium-cum-Workshop" Sulfur in Balanced Fertilization, 4–5 October 2006," (R. K. Tewatia, R. S. Chaudhary, and S. P. Kalwe, Eds.), pp. 1–7. The Sulfur Institute, Washington, DC, The Fertilizer Association of India, New Delhi and International Fertilizer Industry Association, Paris.

Mosier, A. R., Syers, J. K., and Freney, J. R. (2004). Nitrogen fertilizer: An essential component of increased food, feed and fibre production. *In* "Agriculture and the Nitrogen Cycle: Assessing the Impact of Fertilizer Use on Food Production and the environment" (A. R. Mosier, J. K. Seyer, and J. R. Freney, Eds.), Vol. 65. pp. 3–15. Scientific Committee on problems of the Environment (SCOPE) Series, Paris, France. Island Press Washington DC.

Muirhead, W. A., Blackwell, J. B., Humphreys, E., and White, R. J. G. (1989). The growth and nitrogen economy of rice under sprinkler and flood irrigation in southeast Australia. *Irrig. Sci.* **10,** 183–199.

Naidu, B. S., Mahadevappa, M., Inamdar, S. S., Mahapatra, K., and Jayaram (1981). Performance of IR36 in Karnataka, India. *Int. Rice Res. Newsl.* **6**(4), 4.

Nambiar, K. K. M. (1994). Soil Fertility and Crop Production under Long-Term Fertilizer Use in India. Indian Council of Agricultural Research, New Delhi, 144 p.

Nene, Y. L. (1966). Symptoms, causes and control of Khaira disease of paddy. *Bull. Indian Phytopathol. Soc.* **3,** 97–101.

Nguyen, H. T., Babu, R. C., and Blum, A. (1997). Breeding for drought resistance in rice: Physiology and molecular genetics considerations. *Crop Sci.* **37,** 1426–1434.

Nie, L., Peng, S., Bouman, B. A. M., Huang, J., Cui, K., Visperas, R. M., and Park, H. K. (2007). Alleviating soil sickness caused by aerobic mono-cropping: Response of aerobic rice to soil over-heating. *Plant Soil* **300,** 185–195.

Nishio, M., and Kusano, S. (1975). Effect of root residues on the growth of upland rice. *Soil Sci. Plant Nutr.* **21,** 91–395.

Nishizawa, T., Ohshima, Y., and Kurihara, H. (1971). Survey of the nematode population in the experimental fields of successive or rotative plantation. *Proc. Kanto-Tosan Plant Protect. Soc.* **18,** 121–122.

Norman, R. J., Wilson, C. E. Jr., and Slaton, N. A. (2003). Soil fertilization and mineral nutrition in US mechanized rice culture. *In* "Rice: Origin, History and Production" (C. W. Smith and R. H. Dilday, Eds.), pp. 31–412. John Wiley, NJ, USA.

Oelke, E. A., and Mueller, K. E. (1969). Influence of water management and fertility in rice growth and yield. *Agron. J.* **61,** 227–230.

Padgham, J. L., Duxbury, J. M., Mazid, A. M., Abawi, G. S., and Hossain, M. (2004). Yield losses by *Meloidogyne graminicola* on lowland rainfed rice in Bangladesh. *J. Nematol.* **36,** 42–48.

Pal, S., Datta, S. P., Rattan, R. K., and Singh, A. K. (2008). Diagnosis and amelioration of iron deficiency under aerobic rice. *J. Plant Nutr.* **31,** 919–940.

Pandey, H. K., and Mitra, B. N. (1971). Effect of depth of submergence, fertilization and cultivation on water requirement and yield of rice. *Exp. Agric.* **7,** 41–248.

Pathak, H., Li, C., and Wasserman, R. (2005). Greenhouse gas emissions from Indian rice field: Calibrations and up-scaling using the DNDC model. *Biogeosciences* **2,** 113–123.

Patrick, W. H. Jr., and Mahapatra, I. C. (1968). Transformation and availability to rice of nitrogen and phosphorus in water-logged soils. *Adv. Agron.* **20,** 323–359.

Pattanayak, S. K., Mukhi, S. K., and Majumdar, K. (2008a). Phosphate management of hybrid rice. *Better Crops-India* **2,** 29–31.

Pattanayak, S. K., Mukhi, S. K., and Majumdar, K. (2008b). Potassium unlocks the potential for hybrid rice. *Better Crops* **92**(2), 8–9.

Pearce, D. M. E., Hall, K. C., and Jackson, M. B. (1992). The effect of oxygen, carbon dioxide and ethylene biosynthesis in relation to shoot extension in seedlings of rice (*Oryza sativa*) and barnyard grass (*Echinochloa oryzoides*). *Ann. Bot.* **69,** 441–447.

Peng, S. B., Bouman, B. A. M., Visperas, R. M., Castaneda, A., Nie, L. X., and Park, H. K. (2006). Comparison between aerobic and flooded rice in tropics, agronomic performance in an eight season experiment. *Field Crops Res.* **96,** 252–259.

Penning de Vries, F. W. T., Jansen, D. M., Ten Barge, H. F. M., and Bakema, A. (1989). Simulation of Ecophysical Processes of Growth in Several Annual Crops. International Rice Research Institute, Los Banos, Philippines.

Peregrina, F., Mariscal, I., Ordonez, R., Gonzalez, P., Terefe, T., and Espejo, R. (2008). Agronomic implications of converter basic slag as a magnesium source on acid soils. *Soil Sci. Soc. Am. J.* **72,** 402–411.

Pester, T. A., Burnside, O. C., and Orf, J. H. (1999). Increasing crop competitiveness to weeds through crop breeding. *J. Crop. Prod.* **2,** 59–76.

Pinheiro, B. da S. (1999). Characteristicas morphologicas da planta relacionadas a produtivadade. *In* "A Cultura do Arroz no Brasil" (N. R. A. Viera, A. B. Santos, dos Sant'Ana, de and E. P. Guimeraes Eds.). Embrapa Arroz e Feijao, Santo Antonio de Goias, Brasil. Go. pp. 116–147.

Pinheiro, B. da S., Castro, E. da M. de, and Guimaraes, C. M. (2006). Sustainability and profitability of aerobic rice production in Brazil. *Field Crops Res.* **97,** 34–42.

Ponnamperuma, F. N. (1972). The chemistry of waterlogged soils. *Adv. Agron.* **20,** 323–359.

Ponnamperuma, F. N. (1981). Some aspects of the physical chemistry of soils.(Institute of Soil, Academica Sinica, Beijing Eds.). "Proceedings of a Symposium on Paddy Soils", pp. 59–94. Springer Verlag, Berlin.

Postel, S. (1997). Last Oasis. Facing Water Scarcity. Norton, New York, 239 pp.

Potrykus, I., Armstrong, G. A., Beyer, P., Bieri, S., Burkhardt, P. K., Chen, H. D., Ghosh, G. C., Biswas Datta, S. K., Futtere, J., Kloti, A., Spangenberg, G., Terada, R., *et al.* (1996). Transgenic indica rice for the benefit of less developed countries: Toward fungal, insect and viral resistance and accumulation of beta-carotene in the endosperm. "Proceedings of the Third International Rice Genetics Symposium, October 1995", International Rice Research Institute, Manila, Philippines.

Power, J. F., and Follet, R. F. (1987). Monoculture. *Soil Sci. Soc. Am. J.* **25,** 78–86.

Prabhu, A. S., and Filippi, M. C. (2002). Ocorrencia do mal-do pe causado por *Gaeumanomyces gamins var. graminis*, uma nova enfermidade em arroz no Brasil. *Fitopatol. Bras.* **27.**

Prabhu, A. S., Fillippi, M. C., and Ribeiro, A. S. (1999). Doencas e seu controle. *In* "A Cultura do Arroz no Brasil" (N. R. A. Viera, A. B. Santos, and E. P. dos Sant'Ana, Eds.), pp. 267–307. Embrapa Arroz e Feijao, Santo Antonio de Goias, Brasil.

Prasad, R. (2005). Rice–wheat cropping systems. *Adv. Agron.* **86,** 255–339.

Prasad, R. (2006). Zinc in soil and in plant, human and animal nutrition. *Indian J. Fert.* **2**(9), 103–119.

Prasad, R. (2007). Phosphorus management in the rice–wheat cropping system of the Indo-Gangetic Plain. *Better Crops-India* **1,** 8–11.

Prasad, R. (2010). Resource conservation techniques and better plant type for sustainability of rice–wheat cropping system in India. *Indian J. Fert.* **6**(5), 20–28.

Prasad, R., and Power, J. F. (1995). Nitrification inhibitors for agriculture, health and environment. *Adv. Agron.* **54,** 233–281.

Prasad, R., and Power, J. F. (1997). Soil Fertility Management for Sustainable Agriculture. CRC-Lewis, Boca Raton, USA, 356.

Prasad, R., and Rajale, G. B. (1972). The influence of nitrification and slow-release nitrogen materials in transformation of fertilizer nitrogen in soils of fluctuating moisture content. *Soil Biol. Biochem.* **4,** 451–457.

Prasad, R., Rajale, G. B., and Lakhdive, B. A. (1971). Nitrification retarders and slow-release nitrogen fertilizers. *Adv. Agron.* **23,** 337–381.

Prasad, S. R., Bagali, P. G., Hittalmani, S., and Shashidhar, H. E. (2000). Molecular mapping of quantitative trait loci associated with seedling tolerance to salt stress in rice (*Oryza sativa* L.). *Curr. Sci.* **78,** 162–164.

Prasad, R., Shivay, Y. S., Kumar, D., Sharma, S. N., and Devakumar, C. (2007). Neem for sustainable agriculture and the environment. *Proc. Natl. Acad. Sci. India* **77B,** 313–330.

Prasertsak, A., and Fukai, S. (1992). Nitrogen availability and water stress interaction on rice growth and yield. *Field Crops Res.* **52,** 249–260.

Price, A., and Courtois, B. (1999). Mapping QTLs associated with drought in rice: Progress, problems and prospects. *Plant Growth Reg.* **29,** 123–133.

Pulver, E., Jaramillo, S., Moreira, S., and Zorilla, G. (2010). Catching the rains. *Rice Today* **9**(3), 14–16.

Quijano-Guerta, C., Kirk, G. J. D., Portugal, A. M., Bartolome, V. I., and McLaren, G. C. (2002). Tolerance of rice germplasm to zinc deficiency. *Field Crops Res.* **70,** 123–130.

Rajale, G. B., and Prasad, R. (1975). Nitrogen and water management for irrigated rice. *Il Riso* **24,** 117–125.

Rajan, S. S., Watkinson, J. H., and Sinclair, A. C. (1996). Phosphate rock for direct application in soils. *Adv. Agron.* **57,** 78–159.

Ram, P. C., Singh, R. B., Singh, A. K., Singh, O. N., Setter, T. L., Singh, R. K., and Singh, V. P. (1996). Environmental and plant measurement requirements for the assessment of drought, flood and salinity tolerance in rice. *In* "Physiology of Stress Tolerance in Rice". (V. P. Singh, R. K. Singh, R. B. Singh, and R. S. Zeigler, Eds.), Proceedings of the International Conference on Stress Physiology of Rice, 28 February–5 March 1994, pp. 44–69. International Rice Research Institute, Los Banos, Philippines.

Rao, A. N., Johnson, D. E., Shivprasad, B., Ladha, J. K., and Mortimer, A. M. (2007). Weed management in direct seeded rice. *Adv. Agron.* **93,** 153–255.

Rattan, R. K., Datta, S. P., Saharan, N., and Katyal, J. C. (1997). Zinc in Indian agriculture. *Fertil. News* **42**(12), 75–89.

Ray, J. D., Yu, L. X., McCouch, S. R., Champoux, M. C., Wang, G., and Nguyen, H. T. (1996). Mapping quantitative trait loci associated with root penetration ability in rice (*Oryza sativa* L.). *Theor. Appl. Genet.* **92,** 627–636.

Rengel, Z. (2001). Genotypic differences in micronutrient use efficiency in crops. *Commun. Soil Sci. Plant Anal.* **32,** 1163–1181.

Roder, W. (2001). Slash-and-burn rice systems in the hills of northern Lao. PDR: Description, Challenges and Opportunities IRRI, Los Banos, Philippines.

Romheld, V., and Marschner, H. (1990). Genotypic differences among graminae species in release of phytosiderophores and uptake of iron. *Plant Soil* **123,** 147–153.

Sah, R. N., and Mikkelsen, D. S. (1989). Phosphorus behaviour in flooded-drained soils. I. Effect on phosphorus sorption. *Soil Sci. Soc. Am. J.* **53,** 1718–1722.

Sah, R. N., Mikkelsen, D. S., and Hafez, A. A. (1989a). Phosphorus behaviour in flooded-drained soils. II. Iron transformation and phosphorus sorption. *Soil Sci. Soc. Am. J.* **53,** 1723–1729.

Sah, R. N., Mikkelsen, D. S., and Hafez, A. A. (1989b). Phosphorus behaviour in flooded-drained soils. III. Phosphorus desorption and availability. *Soil Sci. Soc. Am. J.* **53,** 1729–1732.

Sahrawat, K. L. (1979). Iron toxicity in an acid sulphate soil as influenced by water regimes. *Plant Soil* **51,** 143–144.

Sakal, R., Verma, M. K., and Singh, A. (1989). Relative susceptibility of selected rice varieties to zinc deficiency in relation to utilization of different pools of zinc from calcareous soil. *Ann. Agric. Res.* **10,** 243–248.

Sanchez, P. A. (1976). Properties and Management of Soils in the Tropics. John Wiley, New York, 618 p.

Sanchez, P., and Uehara, G. (1980). Management considerations for acid soils with high phosphorus fixing capacity. *In* “The Role of Phosphorus in Agriculture” (F. E. Khaswaneh, E. C. Sample, and E. J. Kampreth, Eds.), pp. 417–514. American Society of Agronomy, Madison, WI.

Santana, M. R. (2010). Shift to rice. *Rice Today* **9**(3), 16.

Sarkar, D., Mandal, B., Sarkar, A. K., Singh, S., Jena, D., Patra, D. P., and Phillips, M. (2006). Performance of boronated-NPK in boron deficient soils. *Indian J. Fert.* **1**(12), 57–59.

Sayre, K. D., and Hobbs, P. R. (2004). The raised-bed system of cultivation for irrigated production conditions. *In* “Sustainable Agriculture and the International Rice–Wheat System” (R. Lal, P. R. Hobbs, N. Uphoff, and D. O. Hanson, Eds.), pp. 337–355. Marcel Dekker, New York.

Scardeci, S. C., Sharma, M. C., Grattan, S. R., Eke, A., Roberts, S. R., Goldman-Smith, S., and Hill, J. (2002). Water management practices can affect salinity in rice fields. *Calif. Agric.* **56,** 184–188.

Sekhon, G. S. (1999). Potassium in Indian soils and crops. *Proc. Indian Natl. Sci. Acad.* **B65,** 83–108.

Sha, T., Molden, D., Sakthivadivel, R., and Seckler, D. (2000). The Global Groundwater Situation: Overview of Opportunities and Challenges International Water Management Institute (IWMI), Colombo, Sri Lanka.

Shannon, M. C. (1997). Adoption of plants to salinity. *Adv. Agron.* **60,** 75–120.

Sharma, P. K. (1989). Effect of periodic moisture stress on water use efficiency in wetland rice. *Oryza* **26,** 252–257.

Sharma, S. N. (1999). Water Management. *In* “A Text Book of Rice Agronomy” (R. Prasad, Ed.), pp. 131–144. Jain Brothers, New Delhi.

Sharma, S. N., and Prasad, R. (1999). Effet of *Sesbania* green manuring and mungbean residue incorporation on profitability and nitrogen uptake of a rice–wheat cropping. *Bioresour. Techol.* **67,** 171–175.

Sharma, S. N., Prasad, R., and Singh, R. K. (2000). Influence of summer legumes in rice–wheat cropping system on soil fertility. *Indian J. Agric. Sci.* **70,** 357–359.

Sharma, P. K., Bhushan, L., Ladha, J. K., Naresh, P. K., Gupta, R. K., Balasubramanian, B. V., and Bouman, B. M. (2002). Crop water relations in rice–wheat cropping under different tillage systems and water management practices in a marginally sodic, medium textured soil. *In* “Water-Wise Rice Production” (B. A. M. Bouman, H. Hengsdijk, B. Hardy, P. S. Bindraban, T. P. Tuong, and J. K. Ladha, Eds.), pp. 223–235. International Rice Research Institute, Los Banos, Philippines.

Sharma, S. N., Shivay, Y. S., Prasad, R., Dwivedi, M. K., Davari, M., and Kumar, S. (2010). Relative efficiency of diammonium phosphate and Mussoorie rock phosphate plus

phosphate solubilising bacteria on productivity and phosphorus balance in rice–potato–mungbean cropping system. *J. Plant Nutr.* **33,** 995–1015.

Shihua, T., and Wanqiang, F. (2000). Nutrient management in the rice–wheat cropping system in the Yagtze river flood plain. *In* "Soil and Crop Management Practices for Enhanced Productivity of the Rice–Wheat Cropping System in the Sichuan Province of China" (P. R. Hobbs and R. K. Gupta, Eds.), pp. 24–32. Rice–Wheat Consortium Paper Series 9, Rice–Wheat Consortium for the Indo-Gangetic Plains, New Delhi.

Shivay, Y. S., Kumar, D., and Prasad, R. (2008a). Effect of zinc enriched urea on productivity, zinc uptake and efficiency of an aromatic rice–wheat cropping system. *Nutr. Cycl. Agroecosyst.* **81,** 229–243.

Shivay, Y. S., Kumar, D., Prasad, R., and Ahlawat, I. P. S. (2008b). Relative yield and zinc uptake by zinc sulphate and zinc oxide coating onto urea. *Nutr. Cycl. Agroecosyst.* **80,** 181–188.

Shoemaker, R. K., McLean, E. O., and Pratt, P. F. (1961). Buffer methods for determining lime requirement of soils with appreciable amounts of extractable aluminium. *Soil Sci. Soc. Am. Proc.* **25,** 274–277.

Shrawat, A. K., Caroll, R. T., DePauw, M., Taylor, G. J., and Good, A. (2008). Genetic engineering of improved nitrogen use efficiency in rice by the tissue-specific expression of alanine aminotransferases. *Plant Biotechnol. J.* **6,** 722–733.

Shrotriya, G. C., and Phillips, M. (2002). Boron in Indian agriculture-retrospect and prospect. *Fertil. News* **47**(12), 95–102.

Shukla, D. N. (2010). Impact of fertilizers on Indian agriculture and rural poverty. *Indian J. Fert.* **6**(7), 12–25.

Silanpaa, M. (1990). Micronutrient assessment at the country level—An international study Food and Agriculture Organization (FAO) of the United Nations, RomeBull. 63.

Singh, M. V. (2001). Importance of sulphur in balanced fertilizer in India. *Fertil. News* **40**(10), 13–35.

Singh, S. B., and Abrol, I. P. (1988). Long term effect of gypsum application and rice–wheat cropping on changes in soil properties and crop yield. *J. Indian Soc. Soil Sci.* **36,** 316–319.

Singh, A. K., and Chinnusamy, V. (2007). Aerobic rice—A success story. *Indian Farming* **57**(8), 7–10.

Singh, Y. P., and Misra, J. P. (1974). Water management systems in paddy. *Indian J. Agron.* **19,** 60–63.

Singh, B. N., Singh, B. P., and Singh, M. K. (1981). Relative tolerance of semi-dwarf indica rice to zinc stress in calcareous soils. *Ann. Agric. Res.* **2,** 27–32.

Singh, A. K., Choudhary, B. U., and Bouman, B. A. M. (2002). Effect of rice establishment methods on crop performance, water and mineral nitrogen. *In* "Water-Wise Rice Production" (B. A. M. Bouman, H. Hengsdijk, B. Hardy, P. S. Bindraban, T. P. Tuong, and J. K. Ladha, Eds.), pp. 237–246. International Rice Research Institute, Los Banos, Philippines.

Singh, K., Sharma, H. C., Sarangi, S. K., and Sudhakar, P. C. (2003). Iron nutrition in rice. *Fertil. News* **48**(2), 21–39.

Singh, S., Malik, R. K., Mongia, A. D., Sharma, R. K., Sidhu, B. S., and Bijai-Singh (2005). Haryana & Punjab. *In* "Agenda Notes. 13th Regional Technical Co-Ordination Committee Meeting, 6–8 February 2005, Dhaka, Bangladesh," pp. 27–29. Rice–Wheat Consortium for the Indo-Gangetic Plain, New Delhi.

Singh, V. K., Tiwari, R., Gill, M. S., Sharma, S. K., Tiwari, K. N., Dwivedi, B. S., Shukla, A. K., and Mishra, P. P. (2008). Economic viability of site-specific nutrient management in rice–wheat cropping. *Better Crops-India* **1,** 16–19.

Singh, S., Chhokar, R. S., Gopal, R., Ladha, J. K., Gupta, R. K., Kumar, V., and Singh, M. (2009). Integrated weed management: A key to success for direct seeded rice in the Indo-Gangetic Plain. *In* "Integrated Crop and Resource Management in the Rice–Wheat

Cropping System of South Asia" (J. K. Ladha, Yadvider-Singh, O. Erenstein, and B. Hardy, Eds.), pp. 262–277. International Rice Research Institute, Los Banos, Philippines.

Sivannapan, R. K. (2009a). Advances in micro irrigation in India. *In* "Micro Irrigation. Proceedings of the Winter School on Micro Irrigation, 2–4 March 2009, New Delhi" (T. B. S. Rajput and Patel Neelam, Eds.), pp. 8–18. Water Technology Centre, Indian Agricultural Research Institute, New Delhi.

Sivannapan, R. K. (2009b). Development of micro irrigation technology in India. *In* "Micro Irrigation. Proceedings of the Winter School on Micro Irrigation, 2–4 March 2009, New Delhi" (T. B. S. Rajput and Patel Neelam, Eds.), pp. 1–7. Water Technology Centre, Indian Agricultural Research Institute, New Delhi.

Slaton, N. A., Wilson, C. E. Jr., Ntamatungiro, S., Norman, R. J., and Boothe, D. L. (2001). Evaluation of zinc seed treatments for rice. *Agron. J.* **93,** 152–157.

Slaton, N. A., Norman, R. J., and Wilson, C. E. Jr. (2005). Effect of zinc sources and application on zinc uptake and grain yield of flooded and irrigated rice. *Agron. J.* **97,** 272–278.

Smyth, J. J., and Cravo, M. S. (1997). Aluminium and calcium constraints to continuous crop production in a Brazilian oxisol. *Agron. J.* **84,** 847–850.

Soriano, I. R., and Reversat, G. (2003). Management of *Meloidogynae graminocola* and yield of upland rice in South-Luzon, Philippines. *Nematology* **5,** 879–884.

Srinivasa Rao, Ch., Subba Rao, A., Rao, K. V., Venkateswarlu, B., and Singh, A. K. (2010). Categorization of districts based on non-exchangeable potassium: Implications in efficient K fertility management in Indian agriculture. *Indian J. Fert.* **6**(7), 40–55.

Srivastava, R. C. (2009). Canal supply based pressurized irrigation in flow based micro irrigation. *In* "Micro irrigation. Proceedings of the Winter School on micro irrigation, 2–4 March 2009, New Delhi" (T. B. S. Rajput and Patel Neelam, Eds.), pp. 90–98. Water Technology Centre, Indian Agricultural Research Institute, New Delhi.

Stone, L. F., Pinheiro, B. da S., and Silveira, P. M. (1990). Sprinkler-irrigated rice under Brazilian conditions. *Int. Rice Com. Newsl.* **39,** 36–40.

Swaminatham, M. S. (1984). Rice in 2000 A.D. Report of National Relevance No. 1 Indian National Science Academy, New Delhi, 23 pp.

Swarnalakshmi, K., Dhar, D. W., and Singh, P. K. (2006). Blue-green algae: A potential biofertilizer for sustainable rice cultivation. *Proc. Indian Natl. Sci. Acad.* **92,** 167–178.

Swarup, A. (2002). Lessons from long-term fertilizer experiments in improving fertilizer use efficiency and crop yields. *Fertil. News* **47**(12), 59–73.

Synder, G. H., and Jones, D. B. (1991). Post-emergence treatment of iron-related rice seedling chlorosis. *Plant Soil* **138,** 313–317.

Takahashi, M., Nakanishi, H., Kawasaki, S., Nishizawa, N. K., and Mori, S. (2001). Enhanced tolerance of rice to low iron availability in alkaline soils using barley nicotinamide aminotransferase genes. *Nat. Biotechnol.* **19,** 466–469.

Takkar, P. N., and Nayyar, V. K. (1981). Preliminary field observation of manganese deficiency in wheat and berseem. *Fertil. News* **26**(2), 22–23.

Tewatia, R. K., Chaudhary, R. S., and Kalwe, S. P. (2007). TSI-FAI-IFA Sulfur Project-salient findings. *In* "TSI-FAI-IFA Sulfur Project-Salient Findings. Proceedings of the TSI-FAI-IFA Symposium-cum-Workshop, Sulfur in Balanced Fertilization, 4–5 October 2006" (R. K. Tewatia, R. S. Chaudhary, and S. P. Kalwe, Eds.), pp. 15–25. The Sulfur Institute, Washington, DC, The Fertilizer Association of India, New Delhi and International Fertilizer Industry Association, Paris.

Tracey, P., Sims, B. D., Heifner, S. G., and Cairns, J. P. (1993). Guidelines for Producing Rice Using Furrow Irrigation. Department of Agronomy, University of Missouri, Columbia, MO.

Tripathi, R. P. (1992). Water management in rice–wheat system. *In* "Rice–Wheat Cropping System" (R. K. Pandey, B. S. Dwivedi, and A. K. Sharma, Eds.), pp. 134–147. Project Directorate of Cropping Systems Research, Modipuram, India.

Tuong, T. P. (2009). Promoting AWD in Bangladesh. *Ripple* **4**(3), 1–2.

Tuong, T. P., and Bouman, B. A. M. (2003). Rice production in water-scarce environments. *In* "Water Productivity in Agriculture: Limits and Opportunities for Improvements" (J. W. Kijne, R. Barker, and D. Molden, Eds.), pp. 53–67. CABI Publishing, UK.

Ushimaru, T., Shibaska, M., and Tsuji, H. (1992). Development of oxygen detoxification system during adaptation to air of submerged rice seedlings. *Plant Cell Physiol.* **33,** 1065–1071.

Ventura, W., and Watanabe, I. (1978). Growth inhibition due to continuous cropping of dryland rice and other crops. *Soil Sci. Plant Nutr.* **24,** 375–389.

Ventura, W., Watanabe, I., Castillo, M. B., and de la Cruz, A. (1981). Involvement of nematodes in the soil sickness of a dryland rice based cropping system. *Soil Sci. Plant Nutr.* **27,** 305–315.

Ventura, W., Watanabe, I., Komada, M., Nishio, M., de la Cruz, A., and Castillo, M. (1984). Soil sickness caused by continuous cropping of upland rice, mungbean and other crops. *IRRI Res. Paper Ser.* **99,** 2–13.

Vig, A. C., Bahl, G. S., and Chand, M. (1999). Phosphorus—Its transformation and management under rice–wheat system. *Fertil. News* **44**(11), 33–46.

Vissuwa, M., Ismail, M., and Yanagihara, S. (2006). Effect of zinc deficiency on rice growth and genetic factors contributing to tolerance. *Plant Physiol.* **142,** 731–741.

Vories, E. D., Counce, P. A., and Keisling, T. C. (2002). Comparison of flooded and furrow irrigated rice on clay. *Irrig. Sci.* **21,** 139–144.

Vosenek, L. A. C. J., and van der Veen, R. (1994). The role of phytohormones in plant stress: Too much or too little water. *Acta Bot. Neerl.* **43,** 91–127.

Walkers, T. W., Kingery, W. L., Street, J. E., Lox, M. S., Odham, J. L., Gerard, P. D., and Han, F. X. (2003). Rice yield and soil chemical properties as affected by precision land levelling in alluvial soils. *Agron. J.* **95,** 1483–1488.

Walters, W., and Bos, B. G. (1999). Irrigation Performance Assessment and Irrigation Efficiency.Ann. Rept. International Institute of Land Reclamation and Improvement (ILRI), Wageningen, The Netherlands.

Wang, G., Mackill, D. G., Bouman, J. M., NcCough, S. R., Champoux, M. C., and Nelson, R. J. (1994). RFLP mapping of genes conferring complete and partial resistance to blast in a durably resistant rice cultivar. *Genetics* **136,** 1421–1434.

Wang, H., Bouman, B. A. M., Zhao, D., Wang, C., and Moya, P. F. (2002). Aerobic rice in northern China—Opportunities and challenges. *In* "Water-Wise Rice Production, Proceedings of the International Workshop on Water-Wise Rice Production" (B. A. M. Bouman, H. Hengsdijk, B. Hardy, P. S. Bindraban, T. P. Tuong, and J. K. Ladha, Eds.), pp. 143–154. International Rice Research Institute, Los Banos, Philippines.

Wang, X., Zhu, J., Mansueto, L., and Bruskiewich, R. (2005). Identification of candidate genes for drought stress tolerance in rice by the integration of a genetic (QTL) map with the rice genome physical map. *J. Zhejiang Univ. Sci.* **6B**(5), 382–388.

WARDA (1996). Annual Report for 1995. West Africa Rice Development Association, Bouake, Cote d, Ivoire.

Weil, R. R., and Holah, Sh.Sh. (1989). Effect of submergence on availability of certain plant nutrients in three Ultisol catenas. *Plant Soil* **114,** 147–157.

Willet, I. R. (1982). Phosphorus availability in soils subjected to short periods of flooding and drying. *Aust. J. Soil Res.* **20,** 131–138.

Woperies, M. C. S., Kropff, M. J., and Tuong, T. P. (1996). Modelling the soil–plant–water balance of rainfed low-land rice. *In* "Physiology of Stress Tolerance in Rice".

(V. P. Singh, R. K. Singh, R. B. Singh, and R. S. Zeigler, Eds.), Proceedings of the International Conference on Stress Physiology of Rice, 28 Februray–5 March 1994, pp. 168–185.

Xing, Y. H., Wang, R., Sun, W., An, J. W., Wang, C. X., Bao, H. J., Gong, L., and Wang, X. Z. (2009). Effect of balanced fertilization on rice nutrient uptake, yield and profit. *Better Crops* **93**(1), 8–9.

Xue, C. Y., Yang, X. G., Bouman, B. A. M., Deng, W., Zhang, Q., Yan, W., Zhang, T., Rouzi, A., and Wang, H. (2008a). Optimizing yield, water requirements, and water productivity of aerobic rice for North China Plain. *Irrig. Sci.* **26,** 459–474.

Xue, C. Y., Yang, X. G., Bouman, B. A. M., Deng, W., Zhang, Q. P., Yang, J., Yan, W. X., Zhang, T. Y., Rouzi, A. J., Wang, H. Q., and Wang, P. (2008b). Effects of irrigation and nitrogen on the performance of aerobic rice in north China. *J. Integrat. Plant Biol.* **50,** 1589–1600.

Yadav, J. S. P. (1972). Water management and irrigation scheduling in relation to rice production. *Oryza* **9,** 77–96.

Yadav, R., Courtois, B., Huang, N., and McLaren, G. (1997). Mapping genes controlling root morphology and root distribution in a doubled-haploid population of rice. *Theor. Appl. Genet.* **94,** 619–632.

Yadav, D. K., Yadav, R., Sujata, and Prabhu, K. V. (2007). Rabi and kharif crop varieties. *Indian Farming* **57**(9), 18–22.

Yang, J. L., You, J. F., Li, Y. Y., Wu, P., and Zgang, S. J. (2007). Magnesium enhances aluminium induced citrate excretion in rice beans (*Vigna umbellate*) roots by restoring plasma membrane H+-ATPhase activity. *Plant Cell Physiol.* **48,** 66–73.

Yoshida, S., Ahu, J. S., and Forna, D. A. (1973). Occurrence, diagnosis and correction of zinc deficiency of lowland rice. *Soil Sci. Plant Nutr.* **19,** 83–94.

Yu, J., Hu, S., Wang, G. K., Li, B., Dang, Y., Dai, L., Zhone, Y., Zhang, X., *et al.* (2002). A draft sequence of the rice genome (*Oryza sativa L.* ssp. *Japonica*). *Science* **269,** 79–92.

Zeigler, R. S., Leong, S. A., and Teng, P. S. (1994). Rice Blast Disease. CAB International, Cambridge, UK, 626 p.

Zhang, J., Zheng, H. G., Attari, A., Pantuwan, G., Nguyen, T., Tripathy, J. N., Sarial, A. K., Robin, S., Babu, R. C., Nguyen, B. D., Sakarung, S., and Blum, A. (2001). Locating genome regions associated with components of drought resistance in rice: Comparative mapping within and across species. *Theor. Appl. Genet.* **103,** 19–29.

Zhang, H., Xue, Y., Wang, Z., Yang, J., and Zhang, J. (2009). An alternate and moderate soil drying regime improves root and shoot growth in rice. *Crop Sci.* **49,** 2246–2260.

Zhao, D. L., Altin, G. N., Bastiaans, L., and Spiertz, J. H. J. (2006). Cultivar weed-competitiveness in aerobic rice: Heritability, correlated traits, and the potential for indirect selection in weed-free environments. *Crop Sci.* **46,** 372–380.

Zheng, H. G., Babu, R. C., Pathan, M. S., Ali, M. L., Huang, N., Courtois, B., and Nguyen, H. T. (2000). Quantitative trait loci fpr root penetration and root thickness in rice: Comparison of genetic backgrounds. *Genome* **43,** 53–61.

CHAPTER FIVE

Drought Tolerance: Roles of Organic Osmolytes, Growth Regulators, and Mineral Nutrients

M. Ashraf,[*,†] N. A. Akram,[*] F. Al-Qurainy,[†] *and* M. R. Foolad[‡]

Contents

Abstract

Drought, the occurrence of a substantial water deficit in the soil or in the atmosphere, is an alarming constraint to crop productivity and yield stability worldwide. It is the leading environmental stress in world agriculture, causing

[*] Department of Botany, University of Agriculture, Faisalabad, Pakistan
[†] Department of Botany and Microbiology, King Saud University, Riyadh, Saudi Arabia
[‡] Department of Horticulture, The Pennsylvania State University, University Park, Pennsylvania, USA

Advances in Agronomy, Volume 111
ISSN 0065-2113, DOI: 10.1016/B978-0-12-387689-8.00002-3

losses in crop yield probably exceeding losses from all other causes combined. Drought stress adversely affects a variety of vital physiological and biochemical processes in plants, leading to reduced growth and final crop yield. Some plant species have evolved mechanisms to cope with the stress, including drought avoidance, dehydration avoidance, or dehydration tolerance. Such adaptive mechanisms are the results of a multitude of morphoanatomical, physiological, biochemical, and molecular changes. Osmoregulation is the most common physiological adaptation, which takes place by reducing cellular water potential via accumulation of a variety of organic and inorganic solutes in the cell. As a consequence, such plants are capable of taking up water from a low water potential medium to sustain normal or near normal physiological processes necessary for growth and development. However, most economically important crop species lack the capability of coping with this type of drought stress, precluding their cultivation under water-limited conditions. Various strategies have been proposed to facilitate crop production under drought conditions, in particular, development of new crop varieties with enhanced drought tolerance. Genetic improvement of crop plants for drought tolerance is a long-term endeavor, which requires, among other things, the availability of genetic sources of tolerance, knowledge of the physiological mechanisms and genetic controls of tolerance traits at different developmental stages, and employment of suitable germplasm screening and breeding protocols. An alternative and quicker strategy to promote plant drought tolerance is exogenous application of various compounds, including organic solutes (organic osmolytes and plant growth regulators) and mineral nutrients. Recently, this strategy has gained considerable attention because of its efficiency, feasibility, and cost- and labor-effectiveness. In this chapter, we review the roles of organic osmolytes, plant growth regulators, and mineral nutrients in plant response to drought stress and discuss their exogenous application in enhancing plant drought tolerance and alleviating the damaging effects of drought stress.

Abbreviations

ABA	abscisic acid
BAP	benzylaminopurine
BL	brassinolide
BRs	brassinosteroids
DI	deficit irrigation
EUW	effective use of water
GA_3	gibberellic acid
GAs	gibberellins
GB	glycinebetaine
H_2O_2	hydrogen peroxide

IAA	indole-3-acetic acid
JA	jasmonic acid
MDA	malondialdehyde
$O_2^{\bullet -}$	superoxide radical
OA	osmotic adjustment
$^{\bullet}OH$	hydroxy radical
P5CR	pyrroline-5-carboxylate reductase
P5CS	pyrroline-5-carboxylate synthetase
PEG	polyethylene glycol
PGRs	plant growth regulators
$RO^{\bullet}$	alkoxy radical
ROS	reactive oxygen species
SA	salicylic acid
TPP	trehalose-6-phosphate phosphatase
WUE	water-use efficiency

1. Introduction

Under both wild and cultivated conditions, plants often experience a multitude of environmental stresses such as drought, salinity, waterlogging, extremes of temperature, and mineral toxicities and deficiencies. Environmental stresses are undoubtedly a major cause of food insecurity in many countries around the world, in particular, in developing countries where there is a major challenge to produce sufficient food. A large proportion of the world agriculture depends on rainfall for irrigation, as good quality water supply is highly limited or unpredictable. In many of such regions, crops are often negatively affected by severe drought. It has been estimated that the countries that generate two-third of the world's agricultural product experience water-deficit conditions on a regular basis (Burger, 2009; Revenga *et al.*, 2000). For instance, China, Australia, many African and South American countries, the Middle East, Central Asia, and many states in the United States often experience severe drought conditions (http://www.globalresearch.ca/index.php?context=va&aid=12252), resulting in significant decline in food grain production. The top two major food crops in the world, wheat and rice, have been most severely affected by drought conditions. For example, wheat production in the drought-hit area of the Middle East and Central Asia has decreased by ~22% in 2009 (de-Carbonnel, 2009), and more than 70 million ha of rice-growing area of the world have been negatively affected by drought stress (Athar and Ashraf, 2009). The projected changes in the climate in the coming years may exacerbate

the adverse effects of drought not only in food crops but also in other economically important crops.

Drought causes a significant reduction in plant water content and cell turgor potential, which, in most cases, results in reduced growth rate and final crop yield (Akram *et al.*, 2007, 2008; Ashraf, 2010; Gomes *et al.*, 2008; Guo *et al.*, 2010; Kamran *et al.*, 2009; Mahmood *et al.*, 2009; Sankar *et al.*, 2008; Wang *et al.*, 2009). Under severe drought conditions, drought sensitive plants may fail to grow and ultimately die. Under less severe conditions, drought stress may affect a number of key physiological and biochemical processes including the ability of plants to acquire water and nutrients, which would directly or indirectly affect plant growth and final crop yield. Similar to many other stresses, drought can cause oxidative stress in plants, under which conditions reactive oxygen species (ROS) such as superoxide radical ($O_2^{\bullet -}$), hydroxy radical ($^{\bullet}OH$), hydrogen peroxide (H_2O_2), and alkoxy radical ($RO^{\bullet}$) are produced (Munne-Bosch and Penuelas, 2003). ROS can damage cell membranes, nucleic acids, and proteins (Ashraf, 2009; Mittler, 2002), causing metabolic imbalances in plants. Drought stress often leads to hormonal imbalances (Bajguz and Hayat, 2009; Farooq *et al.*, 2009), changes in activities of enzymes responsible for regulation of key metabolic processes (Tűrkan *et al.*, 2005); and modulation of signal transduction (Chaves *et al.*, 2003), gene expression (Denby and Gehring, 2005), respiration (Ribas-Carbo *et al.*, 2005), and photosynthesis (Flexas *et al.*, 2004).

Although plants undergo a variety of morphoanatomical, physiological, biochemical, and molecular changes in response to water deficit, one of the most common responses is cellular osmotic adjustment (OA), which, if successful, may enable plants to thrive under water-stress conditions (Blum, 2005). OA takes place by accumulation of a variety of organic and inorganic solutes in cells, enabling plants to absorb sufficient amount of water from its external medium to sustain normal functioning of metabolic processes and hence growth (Blum, 2005; Chimenti *et al.*, 2006; Taiz and Zeiger, 2006). Simultaneously, plants produce a variety of antioxidants that counteract the generation of ROS in response to drought stress (Munne-Bosch and Penuelas, 2003; Wang *et al.*, 2009). These include nonenzymatic antioxidants such as tocopherols, carotenoids, ascorbic acid, glutathione, and phenolics, as well as enzymatic antioxidants such as superoxide dismutase, catalase, and enzymes of the ascorbate/glutathione cycle (Alscher *et al.*, 2002; Jaleel *et al.*, 2008; Munne-Bosch and Penuelas, 2003). Water-use efficiency (WUE) is another important plant adaptation under water stress, which also has been proposed as an effective selection criterion to identify and/or develop plants with better drought tolerance (Cao *et al.*, 2007; Ray *et al.*, 2004). In summary, various responses and adaptations are evolved that enable plants sustain growth and development under water-limited conditions.

Table 1 Organic osmolytes, plant growth regulators, and inorganic nutrients commonly applied exogenously to enhance plant drought tolerance

Organic osmolytes	Plant growth regulators	Inorganic nutrients
Glycinebetaine, proline, trehalose, mannitol, sorbitol, glycerol	Auxin, 2,4 dichlorophenoxy acetic acid (2,4-D), indole-3-acetic acid (IAA), gibberellic acid (GA_3), abscisic acid (ABA), ethylene, polyamines (putrescine, spermidine, and spermine), benzylaminopurine (BAP), ethrel, jasmonic acid, salicylic acid, ascorbic acid, brassinolide (BL)	Nitrogen, phosphorus, potassium, calcium, zinc, magnesium, manganese

A variety of strategies have been considered to avert the adverse effects of drought stress in plants. Among them are control of evapotranspiration to counter undue water loss (Taji *et al.*, 2002), use of natural and synthetic conditioners to retain soil moisture content (Huttermann *et al.*, 1999; Sarvas *et al.*, 2007), exploitation of deficit irrigation (DI) strategy to maximize water utilization, effective use of water (EUW) for crop yield improvement (Blum, 2009; Geerts and Raes, 2009), growing drought-hardy plant species, and genetic improvement of drought tolerance in established crops (Bernier *et al.*, 2009; Kumar *et al.*, 2008; Levi *et al.*, 2009). However, an alternative strategy to enhance drought tolerance of commercial cultivars is exogenous application of various compounds, including organic solutes, growth regulators, and mineral nutrients (Ali *et al.*, 2007, 2008; Chen and Murata, 2002; Hussain *et al.*, 2008; Ma *et al.*, 2006, 2007; Mahmood *et al.*, 2009; Rontein *et al.*, 2002) (Table 1). Although each strategy has its own advantages and disadvantages, exogenous application of tolerance-promoting agents has recently gained considerable attention due to being economical, efficient, and relatively less labor-intensive than genetic approaches. Here, we review and discuss the roles of organic osmolytes, plant growth regulators (PGRs), and mineral nutrients in plant response to drought stress, and the implication of their exogenous application in enhancing plant drought tolerance and alleviating the damaging effects of drought stress.

2. Role of Organic Osmolytes in Plant Growth and Metabolism Under Drought Stress

OA capability has been considered as an indirect selection criterion in various breeding programs to develop plants with enhanced drought tolerance (Ludlow and Muchow, 1990; Morgan, 1983; Zhang *et al.*, 1999). In

general, most bacteria, algae, and plants are capable of accumulating a variety of organic solutes in response to osmotic stress (Ashraf *et al.*, 2008; Cushman, 2001; Kufner and Koch, 2008; Liu *et al.*, 2006; Roberts, 2005). The organic solutes, collectively referred to as compatible osmolytes or compatible solutes, not only contribute to osmoregulation but they may also protect the structure of different biomolecules and membranes (Murata *et al.*, 1992; Yancey *et al.*, 1982), or act as free-radical scavengers that protect DNA from damaging effects of ROS (Akashi *et al.*, 2001; Ashraf, 2009).

Glycine betaine (GB), a quaternary ammonium compound, is considered a strong osmoprotectant against multiple stresses, including drought (Ashraf and Foolad, 2007; Chen and Murata, 2002). The specific features of GB that make it an effective metabolite for alleviating stress effects include its small size, solubility in water, and not interfering with other metabolites within the cell. It is nontoxic, even at high concentrations. Further, when exogenously applied as a foliar spray, GB can easily penetrate through leaf epidermis and move to other organs to effectively contribute to enhanced stress tolerance (Makela *et al.*, 1998). Inside plant, the structure of GB is highly stable (Bray *et al.*, 2000). The main route of GB biosynthesis is in the chloroplast, and it includes a two-step oxidation of choline to betaine through betaine aldehyde (Hanson and Scott, 1980; Rhodes and Hanson, 1993). These two biosynthetic steps are catalyzed by choline monooxygenase and betaine aldehyde dehydrogenase. The major function of GB in plant cell under osmotic stress is to stabilize the structure of enzymes, complex proteins, and membranes (Chen and Murata, 2002). GB also promotes cell division (Akula *et al.*, 2000) and protects mitochondrial complex II under salt stress conditions (Hamilton and Heckathorn, 2001). Further, intracellular accumulation of GB allows water retention in the cell and prevents its dehydration (Henke *et al.*, 1997; Rees *et al.*, 1993; http://en.wikipedia.org/wiki/Betaine#Glycine_betaine, 2009).

Amino acid proline is another important osmoprotectant contributing to OA. Proline accumulates in the cytoplasm of many plant species in response to environmental stress, and it plays a significant role in alleviating the adverse effects of stress with varying degrees in different plant species (Ashraf and Foolad, 2007; Hsu *et al.*, 2003; Kavi Kishor *et al.*, 2005; Rhodes *et al.*, 1999). Glutamic acid is the precursor in proline biosynthesis, and pyrroline-5carboxylate synthase (P5CS) and pyrroline-5-carboxylate reductase (P5CR) are two key enzymes of proline biosynthetic pathway (Hu *et al.*, 1992). Proline can stabilize the structure of membranes and proteins, scavenge ROS, and regulate cytoplasmic pH under stress conditions (Bohnert and Shen, 1999; Guo *et al.*, 2010; Kaul *et al.*, 2008; Rodriguez and Redman, 2005; Vanrensburg *et al.*, 1993). Further, it can function as a protein compatible hydrotrope (Kaul *et al.*, 2008). Although the role of proline as a compatible solute in stress tolerance has been greatly investigated (McCue and Hanson, 1990; Yancey *et al.*, 1982), little effort has been devoted to the elucidation of

its active role in other important physiological and biochemical processes mediating stress tolerance (Khedr *et al.*, 2003; Okuma *et al.*, 2000).

Plants also respond to drought stress by accumulating soluble sugars. Sugars are readily available organic osmolytes in the cell. The most common soluble sugars in the cell are sucrose, glucose, and fructose (Taiz and Zeiger, 2006). In addition to these simple sugars, many plants, in particular, resurrection plants accumulate trehalose, when exposed to drought stress. Generally, resurrection plants can accumulate trehalose in the amount up to 1% of their dry weight under nonstress conditions. However, accumulation of this sugar in most crop plants is generally very low (Garg *et al.*, 2002). If trehalose accumulates abundantly in a plant, it can act as an osmolyte and a vital osmoprotectant (Djilianov *et al.*, 2005; Elbein *et al.*, 2003). It is a rich source of carbon and energy (Elbein *et al.*, 2003). It can stabilize membranes and proteins in cells exposed to osmotic stress (Djilianov *et al.*, 2005; Elbein *et al.*, 2003; Han *et al.*, 2005). Its active roles in repressing programmed cell death (Yamada *et al.*, 2006) and scavenging ROS (Elbein *et al.*, 2003) have been demonstrated. Trehalose can also function as a signaling molecule or a regulator of plant growth and development (Avonce *et al.*, 2004; Elbein *et al.*, 2003). Like sucrose, trehalose is also found in the cytosol. Three independent pathways for the biosynthesis of trehalose are known. The most common one involves the key enzyme trehalose-phosphate synthase, which links uridine diphosphate-glucose (UDP-glucose) with glucose-6-phosphate producing trehalose-6-P (a trehalose precursor) and UDP (Elbein *et al.*, 2003). The trehalose-6-P is then converted to trehalose by trehalose-6-phosphate phosphatase (TPP).

During osmotic stress, soluble sugars are produced by hydrolysis of common carbohydrates (Levitt, 1980). An example of this type of conversion is in the resurrection plant *Craterostigma plantagineum* in response to osmotic stress: while well-watered plants accumulate high amounts of 2-octulose (an eight carbon carbohydrate), in plants under osmotic stress, octulose is converted to sucrose (Norwood *et al.*, 2000). This type of carbohydrate transformation is a common phenomenon in many drought-tolerant plants (Peterbauer and Richter, 2001; Prado *et al.*, 2000).

Polyols (polyhydric alcohols) are another important group of widely distributed compatible solutes (Djilianov *et al.*, 2005; Singh *et al.*, 2005; Taravati *et al.*, 2007; Zou *et al.*, 2002). Polyols are generally of two types, acyclic and cyclic. Both acyclic, including glycerol, sorbitol, and mannitol, and cyclic forms, including *myo*-inositol, pinitol, and ononitol, are widely present in plants. Unlike *myo*-inositol, sorbitol and mannitol are direct products of photosynthesis, particularly in fully expanded leaves, just like sucrose (Noiraud *et al.*, 2001). Polyols normally accumulate in the cytosol and counteract the adverse effects of multifarious stresses on metabolism (Ashraf and Harris, 2004). They play an active role in osmoregulation. For example, in transgenic tobacco plants, mannitol alone contributed to 30–40% of the

changes in tissue osmotic potential (Karakas *et al.*, 1997). In addition to their role in osmoregulation, both acyclic and cyclic polyols effectively scavenge ROS generated by various stresses including osmotic stress (Smirnoff and Cumbes, 1989). Due to the putative role of polyols as antioxidants, their role in the regulation of plant metabolism appears to be of more significance than their osmotic role (Griffin *et al.*, 2004). In yeast (*Saccharomyces cerevisiae*), polyols may have a dual function in stress protection, acting in both OA and regulation of the redox system within cells (Shen *et al.*, 1999). Polyols also can translocate energy and carbon compounds between the source and the sink organs. Improved transport of polyols has been observed in both xylem and phloem in response to salt or drought stress (Noiraud *et al.*, 2001).

In summary, compatible solutes play major roles in plant stress tolerance. Their principal role is maintenance of osmoregulation (turgor) in plants exposed to stress conditions. The maintenance of turgor is in fact crucial for maintaining normal cell activity under water-limited conditions. In addition, compatible osmolytes play significant roles in other cellular functions such as stabilization of proteins, protection of membrane structure, and scavenging of ROS. However, accumulation of the various types of compatible solutes varies from species to species. As a general rule, plants that accumulate high concentrations of organic osmolytes normally exhibit a greater tolerance to drought stress, compared to plants that accumulate lower or negligible amount of such solutes. Efforts are underway to develop drought-tolerant plants by producing transgenics with enhanced synthesis/accumulation of compatible organic osmolytes. However, a few reports have indicated that high accumulation of osmolytes in genetically engineered plants have caused impaired growth, particularly in the absence of a stress. Most likely, this may occur because of an adaptation strategy in plants to conserve water under no stress to be prepared for future potential stress conditions (Abebe *et al.*, 2003; Maggio *et al.*, 2002). Thus, further fine-tuning of the synthesis of osmolytes in transgenic plants is essential in such genetic transformation studies. However, a different strategy to improve plant drought tolerance is exogenous application of such osmolytes, as discussed below.

3. Exogenous Application of Organic Osmolytes for Improving Plant Drought Tolerance

Organic osmolytes can be externally applied through three different means; as a presowing seed treatment, through the rooting medium, or as a foliar spray. The application of osmolytes through the soil (growth medium) does not seem to be feasible because supplemented osmolytes are prone to degradation by soil microorganisms (Ashraf *et al.*, 2008). Further, large-scale use of osmolytes as soil additives to ensure adequate supply to plants is very

costly. Thus, application of osmolytes either as presowing seed treatment or foliar spray is preferable. In comparison with foliar application, presowing seed treatment is more cost-effective, provided its effects are long lasting in improving plant growth and metabolism throughout plant ontogeny.

3.1. Presowing seed treatment with organic osmolytes

Rapid and uniform seed germination and seedling emergence are important determinants of successful stand establishment and crop development (Murungu *et al.*, 2003). However, under water-limited conditions, poor germination and seedling emergence are major constraints to crop establishment. Insufficient water during seed germination may lead to poor seed germination and hence poor crop establishment. To improve seed germination and emergence and stand establishment under water-limited conditions, presowing seed treatments (priming) of various types have been considered (Atreya *et al.*, 2009; Harris, 1996; Harris *et al.*, 1999, 2001, 2002; Yagmur and Kaydan, 2008). During priming, seeds are allowed to imbibe water and begin some of the key metabolic processes related to the initiation of germination. Emergence of the radicle is avoided to prevent the loss of dehydration tolerance that is essential for storage and marketing of the primed seed (Soeda *et al.*, 2005). Primed seed generally germinate faster and more uniformly than unprimed seed when they are subjected to an appropriate germination milieu (Ashraf *et al.*, 2005; Ghana and William, 2003; Guan *et al.*, 2009). During the past few decades, seed priming has been widely employed to improve the rate and uniformity of seed germination and emergence under water-limited conditions in many commercially important crop plants (Atreya *et al.*, 2009; Harris *et al.*, 1999, 2001, 2002; Yagmur and Kaydan, 2008). There are different techniques of seed priming, including hydropriming, osmopriming, halopriming, hormone priming, and thermopriming (Ashraf and Foolad, 2005; Ashraf *et al.*, 2008). Although all these techniques are economical, simple, and efficient for improving seed germination and crop establishment under stressful conditions (Kaur *et al.*, 2002), the focus of the present review is on osmopriming.

Osmopriming refers to soaking seed in solutions of sugars, sugar alcohols, or polyethylene glycol (PEG), followed by surface drying of the seed to some low and stable moisture level. In this process, the low water potential of the priming solution allows partial seed hydration, thereby allowing the initiation of some pregermination metabolic processes in the seed, but just below the level needed for germination to occur. When planted in the field, the osmoprimed seeds generally show rapid and uniform germination and emergence when they come in contact with soil moisture (Ashraf and Foolad, 2005; Ashraf *et al.*, 2005; McDonald, 2000; Pill and Necker, 2001). This is a routine practice in many agriculturally important plant species for improving seed germination under stress or

nonstress conditions. For example, in chickpea (*Cicer arietinum* L.), seed primed at varying levels of PEG (−0.5, −1.0, −1.5, and −2.0 MPa) or in 4% solution of mannitol exhibited fast and uniform germination under varying temperatures (Elkoca *et al.*, 2008). Naidu *et al.* (1998) conducted a series of rainfed field experiments with cotton (*Gossypium hirsutum* L.) plants raised from seed coated with 5% or 7.5% GB. In this research, a substantial increase in the yield of cottonseed was observed under water-deficit conditions due to the priming treatment. Similarly, osmopriming of rice seed with GB resulted in a marked improvement in growth under water-deficit regimes measured as seedling fresh and dry weights (Farooq *et al.*, 2008). This GB-induced improvement in growth was determined to be associated with enhanced plant photosynthetic capacity and upregulation of antioxidative defense system. There are, however, a few reports of either negative or no effect of osmopriming on growth and metabolism in some plant species. For example, wheat seed osmoprimed with PEG exhibited lower germination rate, vigor index, and seedling dry weight compared to nonprimed seed sown under dry conditions (Ahmadi *et al.*, 2007). And presowing treatment of sunflower (*Helianthus annuus* L.) seed with 50 or 100 mM of GB did not affect any of the water relation attributes such as osmotic, turgor, and water potentials measured under either well-watered or water-deficit conditions (Iqbal *et al.*, 2008).

A cDNA microarray analysis in cabbage (*Brassica oleracea* L.) revealed that a number of genes were upregulated in the seed during osmopriming, confirming the premise that germination-related biochemical processes are initiated during the priming treatment (Soeda *et al.*, 2005). However, before more reports are available on the exact effects of osmopriming on germination, growth, metabolic processes, and gene expression in seeds subjected to drought stress, it is not possible to infer how osmopriming affects plant drought tolerance. Nonetheless, in the majority of cases, it is evident that osmoprimed seeds generally exhibit accelerated and uniform germination in comparison to nonprimed seeds under drought stress. Seed priming has been employed for many years in different plant species, not only to improve the rate and uniformity of seed germination and synchronize seedling emergence and establishment under normal field conditions but also to enhance crop productivity under water-limited environments. The consensus is that, similar to other presowing seed treatments, osmopriming has a long-lasting effect on plant growth and development leading to higher crop yield.

3.2. Foliar application of organic osmolytes

The main advantage of foliar spray over presowing seed treatment is that osmolytes can be applied at any critical stage when their availability could most effectively influence plant growth and productivity. Also, foliar application of osmolytes or nutrients is beneficial in situations where solute

deficiencies could not be remedied by soil amendment. In general, plant species differ in their natural ability to synthesize and accumulate osmolytes (Ashraf and Foolad, 2007; Farooq *et al.*, 2008; Iqbal *et al.*, 2008). For example, some nonaccumulator species are not capable of producing certain organic osmolytes such as GB and proline (Ashraf and Foolad, 2007). Foliar application of osmolytes in nonaccumulators may prove to be highly beneficial in improving plant's ability to cope with stress environment (Allard *et al.*, 1998; Harinasut *et al.*, 1996; Iqbal *et al.*, 2008). There are several reports of the beneficial effects of foliar application of osmolytes in improving plant growth and final crop yield under water-stress conditions. For example, in tobacco plants grown under water stress, foliar application of GB (100 or 300 mM) resulted in substantial increases in leaf biomass and total leaf area (Agboma *et al.*, 1997). Similarly, foliar application of GB (100 mM) to water-stressed sunflower plants resulted in marked improvements in several traits, including capitulum diameter, number of achenes, achene weight, total achene yield, and achene oil content (Hussain *et al.*, 2008). The treated plants also showed higher accumulations of GB and proline in the leaf, compared to nontreated plants. In common beans (*Phaseolus vulgaris* L.), a slower drop in leaf water potential was observed in GB-treated as compared to untreated plants grown under water stress (Xing and Rajashekar, 1999). The treated bean plants recovered more rapidly from drought-caused wilting following the termination of drought stress, compared to the untreated plants. In rice plants, growth reduction due to water stress was highly reversed by foliar application of GB (Farooq *et al.*, 2008). This effect was attributed to GB-induced improvement in water relations, antioxidant system, and photosynthetic capacity (Table 2).

The effectiveness of foliar-applied organic osmolytes may differ at different stages of plant development, and thus the most suitable stage for application must be determined. The foliar application of GB to sunflower plants to improve achene yield under water stress was most effective when applied during reproductive stage, though application during vegetative stage was also effective in alleviating the inhibitory effects of drought stress (Iqbal *et al.*, 2008). Similarly, Hussain *et al.* (2008) determined that the greater benefits of GB in sunflower were when it was applied at the flowering stage. In contrast, Ali *et al.* (2008) discovered positive effects of exogenous proline in counteracting the adverse effects of drought stress regardless of the growth stage at which it was applied.

The effectiveness of an osmolyte in improving plant growth under water-stress conditions depends on the nature of osmolyte as well as the plant species under study (e.g., accumulators vs. nonaccumulators). Generally, plants lacking the intrinsic ability to accumulate sufficient level of a specific osmolyte are more responsive to exogenous supply of the osmolyte, compared to plants that naturally accumulate high concentrations of it. Also, for each plant species, the effective level of an osmolyte and the frequency of

Table 2 Improvement in growth and regulation of various physiological and biochemical processes in different plant species by exogenous application of organic osmolytes under drought stress

Organic osmolyte	Mode of application	Concentration applied	Level of drought stress used	Species	Response	Reference
Proline	Foliar spray	30 and 60 mM	60% field capacity	Maize (*Zea mays* L.)	Improved growth, photosynthetic rate, stomatal conductance, substomatal CO_2 concentration, and chlorophyll content	Ali *et al.* (2007)
	Foliar spray	30 and 60 mM	60% field capacity	Maize	Enhanced accumulation of essential nutrients such as K^+, Ca^{2+}, N, and P	Ali *et al.* (2008)
	Presowing seed treatment	20 and 40 mM	60% field capacity	Common wheat (*Triticum aestivum* L.)	Improved shoot and root fresh and dry weight, shoot length, grain yield, and total leaf area per plant	Kamran *et al.* (2009)
	Foliar spray	20 mg L^{-1}	Soil matric water potential maintained at −0.03, −0.5, −1.0, and −1.5 MPa	Cotton (*Gossypium barbadense* L.)	Enhanced chlorophyll contents, chlorophyll stability index, leaf relative water content, and dry matter accumulation	Gadallah (1995)

Glycinebetaine (GB)	Foliar spray	100 mM	One irrigation was less as compared to control	Sunflower (*Helianthus annuus* L.)	Enhanced proline and GB contents	Hussain *et al.* (2008)
	Seed priming and foliar spray	50, 100, and 150 mg L^{-1}	50% field capacity	Rice (*Oryza sativa* L.)	Improved growth under well-watered and water-deficit conditions due to enhanced water potential, antioxidant system, integrity of cellular membranes, and photosynthesis	Farooq *et al.* (2008)
	Foliar spray	100 mM	10%, 18%, and 25% lower relative water content (RWC)	Common wheat	Improved photosynthetic rate, photochemical activity and efficiency of PSII (F_v/F_m), prevented photoinhibition, and improved antioxidative system	Ma *et al.* (2006)
	Foliar spray	80 mM	50% field capacity	Tobacco (*Nicotiana tabacum* L.)	Enhanced growth, photosynthesis, stomatal conductance, activity of photosystem II (PSII), and antioxidative enzyme activities, and maintained water potential and osmotic adjustment under water-deficit conditions	Ma *et al.* (2007)

(*Continued*)

Table 2 (*Continued*)

Organic osmolyte	Mode of application	Concentration applied	Level of drought stress used	Species	Response	Reference
	Presowing treatment	50 and 100 mM	60% field capacity	Common wheat	Increased shoot fresh biomass and leaf area per plant	Mahmood *et al.* (2009)
	Foliar spray	0.1 and 0.3 mM	36% soil water contents	Tobacco	A considerable increase in leaf fresh and dry weights, leaf area, and glycinebetaine contents	Agboma *et al.* (1997)
	Presowing seed treatment	2.5%, 5.0%, and 7.5% (w/w)	5% soil moisture	Cotton (*Gossypium hirisutum L.*)	High GB accumulation and 18–22% increased seed cotton yield at 5% and 7.5% GB levels	Naidu *et al.* (1998)
	Foliar spray	50 and 100 mM	60% field capacity	Sunflower	Enhanced leaf water and turgor potentials and achene yield per plant under drought stress	Iqbal *et al.* (2008)
	Foliar spray	50 and 100 mM	60% field capacity	Sunflower	Improved achene weight but plant growth was not affected	Iqbal *et al.* (2005)
	Foliar spray	100 mM	45.9% water contents in soil	Common wheat	Improved Ca^{2+}-ATPase, Hill reaction activities, chlorophyll content, gas exchange characteristics, and lipid composition of thylakoid membranes	Zhao *et al.* (2007)
	Foliar spray	100 mM	50% field capacity	Common wheat	Improved antioxidant enzyme activity	Ma *et al.* (2004)

application need to be determined in order to achieve the highest improvement in performance under water-stress conditions. It is also important to determine the growth stage at which exogenous application of an osmolyte is most effective, as this may differ among plant species. Further, the diurnal timing of the application may have an impact on its effectiveness. For example, during the evening hours of cool temperature and high humidity, application may be more effective due to minimized evaporation and maximized leaf contact, and thus better penetration of the solute through epidermal cells. Moreover, proper choosing of a chemical surfactant is necessary, as this would enhance the penetration of osmolyte solution. Thus, consideration and optimization of all these contributing factors are expected to enhance the effectiveness of exogenous application of osmolytes under field conditions.

4. Role of Plant Growth Regulators in Drought Tolerance

PGRs are actively involved in a multitude of metabolic processes and play essential roles in plant growth and development under both stress and nonstress conditions. They also act as chemical messengers to modulate various processes or genes involved in plant growth and development (Morgan, 1990). PGRs also play important roles in plant adaptation to stressful environments, including drought stress (Huang *et al.*, 2008). There are two classes of PGRs, growth promoters and growth retardants. However, all major PGRs, including auxins, gibberellins (GAs), cytokinins (all growth promoters), abscisic acid (ABA; a growth retardant), and ethylene (a gaseous hormone known for its roles in plant maturation) are directly involved in regulation of water movement at the root and shoot levels by altering the permeability of cell membranes and ultimately cell turgor (Giulivo *et al.*, 1985).

Plant growth retardants have been determined to play significant roles in plant growth and development as well as plant adaptation to stress environment (Hajihashemi *et al.*, 2009; Jaleel *et al.*, 2007, 2008; Manivannan *et al.*, 2007, 2008; Marshall *et al.*, 2000). Triazole compounds, including fungicides triadimefon (TDM), ketoconazole (KCZ), paclobutrazol (PBZ), and propiconazole (PCZ), have been recognized as plant growth retardants (Fernández *et al.*, 2006; Jaleel *et al.*, 2007, 2008; Manivannan *et al.*, 2007, 2008; Percival and Noviss, 2008; Sankar *et al.*, 2007). These compounds regulate plant growth and development mainly not only by regulating isoprenoid pathway, but also by altering the levels of certain plant hormones, including reduction in gibberellin biosynthesis and ethylene evolution, increasing cytokinin concentration (Kamountsis and Chronopoulon-

Sereli, 1999), improving free-radical scavenging system (Kopyra and Gwozdz, 2003), and promoting alkaloid production (Jaleel *et al.*, 2006). PBZ, a growth retardant, has been examined for improving drought tolerance in plants (Fernández et al., 2006; Marshall *et al.*, 2000). PBZ has antagonistic effects against plant hormone gibberellin, including inhibition of gibberellin biosynthesis, reducing internodial growth, increasing root growth, causing early fruitset, and increasing seedset (Berova and Zlatev, 2000; Grossi *et al.*, 2005). Further, PBZ has been used to reduce shoot growth and shown to have additional positive effects on trees and shrubs, including improved resistance to drought stress, higher resistance against fungi and bacteria, and enhanced development of roots (Chaney *et al.*, 1996).

Among PGRs with retardant effects, ABA is known to play major roles in regulating downstream responses to various environmental stresses. For example, it acts as an effective signal to induce stomatal closure to control water loss under dry conditions (Davies and Zhang, 1991). In response to drought stress, ABA is synthesized in the root, transported to the leaves via the xylem, and accumulated in the guard cells to induce stomatal closure (Taiz and Zeiger, 2002). Slight alterations in ABA distribution may set off stomatal closure initially, and the closure thereafter is maintained by a consistent increase in endogenous level of ABA (Morgan, 1990). Numerous molecular and genomic studies have revealed that stress-responsive gene expression is mediated by both ABA-dependent and ABA-independent control systems (Bray, 1997; Riera *et al.*, 2005; Shinozaki and Yamaguchi-Shinozaki, 1997, 2000). For example, in *Arabidopsis* [*Arabidopsis thaliana* (L.) Heynh], it was determined that expressions of ~14% of the genes were modulated by ABA (Huang *et al.*, 2007). Using oligonucleotide microarray analysis, Huang *et al.* (2008) determined that ~2000 genes in *Arabidopsis* were drought responsive, of which the expression of two-third was regulated by ABA and/or ABA analogues. Further, this study indicated that plant hormones such as auxins, GAs, cytokinins, ethylene, brassinosteroids (BRs), and jasmonic acid (JA) were also actively involved in drought-related gene expression, of which JA was the most involved.

JA, 3-oxo-2-(2-*cis*-pentenyl cyclopentane-1 acetic acid), and its methyl ester (methyl jasmonate, MJ) are naturally occurring PGRs, which affect various physiological and biochemical processes in plants (Fonseca *et al.*, 2009; Jubany-Mari *et al.*, 2010; Wang, 1999). Jasmonates are biologically similar to ABA and generally inhibit stomatal opening, cell division, plant growth, photosynthetic activities, flower bud formation, seed germination, and embryogenesis (Creelman and Mullet, 1997; Koda, 1992; Sembdner and Parthier, 1993; Yamane *et al.*, 1981). However, both JA and MJ enhance and/or induce leaf senescence, petiole abscission, fruit ripening, chlorophyll degradation, carotenoid biosynthesis, tuber formation, and protein synthesis (Davies *et al.*, 1986; Creelman and Mullet, 1997). Further, when exogenously applied, JA and MJ elicit a wide variety of

morphological and physiological processes. For example, while examining the effect of methyl MJ on changes in oxygen-scavenging enzyme activities and membrane lipid composition in strawberry leaves under water stress, Wang (1999) discovered that it reduced the peroxidase activity, maintained higher catalase and superoxide dismutase activities, increased MDA (malondialdehyde) and ascorbic acid content, and reduced transpiration and degree of fatty acid unsaturation.

Cytokinins are actively involved in the regulation of numerous biological processes during plant growth and development. For example, they can induce the formation of and protect cellular structures (Chiappetta *et al.*, 2006), induce and accelerate protein synthesis (Chernyadev, 2005), and induce the opening of stomata thereby increasing stomatal conductance and transpiration (Jewer *et al.*, 1985; Lechowski, 1997). Cytokinins also are involved in regulation of plant responses to abiotic stresses, including drought stress (Haberer and Keiber, 2002; Rivero *et al.*, 2007). ABA and cytokinins exhibit antagonistic effects on plants grown under water-deficit conditions. For example, a decrease in cytokinin and an increase in ABA content under water stress promote stomatal closure thereby reducing water loss (Goicoechea *et al.*, 1997; Morgan, 1990). Havlova *et al.* (2008) reported that in tobacco plants grown under drought-stress conditions, ABA levels markedly increased, particularly in upper leaves, whereas the bioactive cytokinin content steadily decreased. This study suggested that low concentrations of cytokinins were useful for the protection of leaves from harmful effects of drought. It was also noted that the levels of both auxins and cytokinins increased in the root in response to drought stress, suggesting that both hormones may play active roles by stimulating primary root growth to extract water and nutrients from deeper soil strata.

Auxins constitute a small group of plant hormones, which play central roles in the upregulation of plant growth and development (Gadallah, 2000; Michalczuk *et al.*, 1992; Sitbon and Perrot-Rechenmann, 1997; Sitbon *et al.*, 1993). Auxins stimulate stomatal opening in the leaves and water movement in the root (Mansfield and Mc-Ainsh, 1995). They act in conjunction with ABA, generally affecting leaf turgor potential under drought- and salt-stress conditions, though there is little evidence of variation in auxin level in water-stressed plants (Lopez-Carbonell *et al.*, 1994; Rimbaut and Pilet, 1994).

GAs are also an important group of endogenous PGRs, which play vital roles in promoting plant growth and development as well as plant response to biotic and abiotic stresses (Huerta *et al.*, 2009; Sun, 2008). Specific roles of GAs include induction of seed germination, promotion of hypocotyl growth and stem elongation, regulation of flower initiation, pollen development, and xylogenesis and xylem fiber elongation (Ritchie and Gilroy, 1998). Auxins and GAs together also play important roles in plant response to drought stress (Grossmann, 2003; Woodward, 2005). For example, reduction in the level of both hormones may lead to inhibition in plant

growth and development observed in different plant species under water-deficit conditions (Aharoni *et al.*, 1977; Guinn and Brummett, 1988).

Ethylene plays important roles in modulating plant responses to abiotic stresses, including drought, salinity, waterlogging, and extremes of temperature. A marked increase in ethylene concentration is generally observed in plants subjected to water deficit. Drought stress-induced enhanced respiration, senescence, and ripening have been determined to be associated with increased ethylene production (Morgan, 1990). Further, lipid peroxidation caused by ROS under water-limited conditions is also related to enhanced ethylene biosynthesis (Hildebrand and Grayburn, 1991; Leshem, 1981; Smirnoff, 1993). However, generally plants that synthesize low levels of ethylene are more tolerant to the injurious effects of environmental stresses as compared to those that synthesize higher levels of it (Alvarez *et al.*, 2003; Leonard *et al.*, 2005; Munné-Bosch *et al.*, 2004). Further, under stress conditions, the relationship between ethylene and other hormones such as ABA is important for the maintenance of growth (Leonard *et al.*, 2005; Picarella *et al.*, 2007). For example, it has been determined that, under stress conditions, at early vegetative growth stages, ethylene acts as a negative regulator of ABA, while in the root it has a positive synergistic effect on ABA action by adjusting carbon status (Ghassemian *et al.*, 2000; Zhou *et al.*, 2006). Generally, under water-stress conditions, the increased endogenous level of ABA limits ethylene production, and as such it maintains proper growth ratio between shoot and the root (Sharp, 2002).

BRs, a relatively new class of PGRs, play various protective and stimulatory roles in growth and development of plants exposed to stressful environments by modulating several metabolic processes leading to stress tolerance (Ashraf *et al.*, 2008; Catala *et al.*, 2007; Khripach *et al.*, 2000; Shahbaz *et al.*, 2008). For examples, BRs can affect cell expansion and elongation, seed germination, rhizogenesis, flowering, senescence, abscission and maturation, vascular differentiation, signal transduction, and delayed senescence (Cano-Delgado *et al.*, 2004; Nemhauser and Chory, 2004). Further, BRs can effectively scavenge active oxygen species by increasing the activity of antioxidant enzymes in plants subjected to stress environments (Ozdemir *et al.*, 2004; Shahbaz *et al.*, 2008).

Polyamines (spermidine, spermine, putrescine, and cadaverine) have been identified as essential endogenous PGRs as well as signal molecules in a variety of physiological responses (Davies, 2004; Yang *et al.*, 2007). Polyamines occur in plants in free and conjugated forms (Gemperlova *et al.*, 2006; Martin-Tanguy, 2001). They play important roles in regulation of a variety of physiological processes, including cell division, morphogenesis, senescence, programmed cell death (apoptosis), and secondary metabolism (Davies, 2004; Kuehn and Phillips, 2005). Their roles in plant protection against biotic and abiotic stresses have also been reported (Galston, 2001; Liu *et al.*, 2006; Ma *et al.*, 2005). Generally, plants possessing high amounts

of polyamines are more stress tolerant than those with low concentrations (Kasukabe *et al.*, 2004), and when plants are exposed to drought stress, the content of polyamines is generally augmented (Capell *et al.*, 2004; Kasukabe *et al.*, 2004; Ma *et al.*, 2005). In contrast to the rather known roles of the above-mentioned naturally occurring PGRs in plant stress tolerance, roles of other plant hormones such as ascorbic acid, tocopherols, triacontanol, and systemin in plant stress tolerance are less defined.

Although each hormone has its own specific mode of action and signal transduction pathway, most of the physiological and biochemical functions are regulated or are dependent on other hormones. For example, though cell division is predominantly mediated by cytokinins, other hormones such as auxins and GAs also affect this process. Similarly, cell elongation is regulated by the function of auxins, GAs, and BRs as well as a few other hormones. Detailed molecular studies have revealed that cross-talks among PGRs are a common phenomenon, which involves changes in the manifestation of hormone-related biosynthetic genes and/or signaling molecules. Such changes have resulted in alterations in the levels of natural hormones (Bajguz and Hayat, 2009; Rock and Sun, 2005). Integration of various physiological and biochemical processes regulated by different hormones is intricate and thus not easy to elucidate. However, similar to other abiotic stresses, drought stress causes alterations not only in the levels of various endogenous PGRs but possibly also in the complicated cross-talks among them.

5. Exogenous Application of Plant Growth Regulators to Improve Drought Tolerance

As eluded to earlier, drought stress can perturb the optimum levels of PGRs, which are actively involved in plant growth and development. Such disturbance would result in slowed growth and reduced final yield under drought stress. Some plants have adapted mechanisms to avoid hormonal imbalances under drought stress, and thus may exhibit resiliency under such conditions. However, in plants which cannot naturally maintain optimum levels of PGRs under the stress, exogenous application of PGRs may overcome their deficiency (Ashraf *et al.*, 2008). Exogenous application of PGRs can be via presowing seed treatment or foliar spray, as discussed below.

5.1. Presowing seed treatment with PGRs

Presowing seed treatment with different PGRs has been identified as an effective approach to improving plant growth and development under water-stress conditions in several plant species. For example, Heikal *et al.* (1982) investigated the presowing treatment of onion (*Allium cepa* L.), flax

(*Linum usitatissimum* L.), and sesame (*Sesamum indicum* L.) seeds with gibberellic acid (GA_3). While such treatment was effective in improving the rate and final percentage of germination under osmotic stress in flax and sesame, a slight negative effect of the treatment was observed in onion. Zeid and Shedeed (2007) reported improved germination in alfalfa (*Medicago sativa* L.) seed under osmotic stress by presowing treatment with polyamine putrescine. Similarly, Farooq *et al.* (2009) demonstrated that presowing treatment of rice kernels with different types of polyamines, including putrescine, spermidine, and spermine, resulted in improved drought tolerance in the plants. However, more research is needed to examine the utility of this approach in improving drought-stress tolerance of major crop species.

5.2. Foliar application of PGRs

PGRs have been applied as foliar spray to alleviate the adverse effects of drought stress in various plant species (Table 3). For example, Pandey *et al.* (2003) examined the effects of foliar-applied PGRs, including indole-3-acetic acid (IAA), GA_3, ABA, benzylaminopurine (BAP), and ethrel on cotton plants grown under drought stress. It was determined that while application of ABA and ethrel resulted in marked reductions in gas exchange characteristics and photosynthetic pigments, application of BAP alleviated the adverse effects of drought stress on these characteristics. Also, ABA treatment alleviated the inhibitory effects of drought stress on cottonseed number and lint mass. Abreu and Munne-Bosch (2008) investigated the effects of exogenous application of SA and JA in perennial common sage (*Salvia officinalis* L.) grown under drought stress and reported that while SA promoted leaf senescence, JA did not have such an effect at least at the low concentrations applied. Amin *et al.* (2009) reported that foliar application of SA or ascorbic acid was effective in mitigating the harmful effects of drought stress and enhancing plant growth in okra (*Hibiscus esculentus* L.). Such effects were attributed to potential antioxidant activities of these PGRs and their ability to scavenge ROS.

Several reports have shown that exogenous application of BRs can effectively alleviate the adverse effects of abiotic stresses in various plant species (Table 3). For example, Zhang *et al.* (2008) evaluated the effectiveness of brassinolide (BL), one of the most common BRs in the plant kingdom, in alleviating adverse effects of drought stress on photosynthesis and antioxidant system in soybean (*Glycine max* L.). They reported that BL treatment (0.1 mg L^{-1}) was effective in improving biomass production and seed yield in soybean plants grown under well-watered or water-deficit conditions. The BL-induced growth improvement in soybean plants under drought stress was determined to be associated with improved net CO_2

Table 3 Regulation of various physiological and biochemical processes in different plant species by exogenous application of plant growth regulators (PGRs) under drought stress

PGR	Mode of application	Concentration applied	Level of drought stress used	Species	Response	Reference
Abscisic acid (ABA)	Foliar spray	200 or 400 mg L^{-1}	Water was withheld for 48, 72, 96, 120, or 144 h	Various annual bedding flowering plants	Increased drought tolerance, postharvest longevity of several bedding plants extended	Blanchard *et al.* (2007)
	Foliar spray	100 μM	Withholding of irrigation	Bermudagrass (*Cynodon dactylon* L.)	Enhanced relative water content and activities of superoxide dismutase (SOD) and catalase (CAT) and increased H_2O_2 and NO contents, while decreased ion leakage and MDA contents	Lu *et al.* (2009)
Salicylic acid and ascorbic acid	Foliar spray	1 mM	1/3% and 2/3% field capacities	Okra (*Hibiscus esculentus* L.)	Improved leaf area, fresh and dry weights of leaves, proline content and mitigated the oxidative damage due to drought stress	Amin *et al.* (2009)
Salicylic acid	Foliar spray	10^{-4} M methyl salicylic acid	Exposed to water deficit by withholding water	Common sage (*Salvia officinalis* L.)	Reduced chlorophyll content, and promoted leaf senescence	Abreu and Munne-Bosch (2008)

(*Continued*)

Table 3 (*Continued*)

PGR	Mode of application	Concentration applied	Level of drought stress used	Species	Response	Reference
	Presoaking	0.5 mM	15% PEG	Maize (*Zea mays* L.) and common wheat (*Triticum aestivum* L.)	Decreased drought tolerance as a result of enhanced electrolyte leakage while decreased net photosynthetic rate	Nemeth *et al.* (2002)
Gibberellic acid and abscisic acid	Foliar spray	10^{-6} M	Withholding of water first after 75 days and second after 88 days of sowing)	Mungbean (*Vigna radiata* L. Wilczek)	Improved yield, weight of pods per plant, and shoot dry weight per plant	Ayub *et al.* (2000)
Indole-3-acetic acid, gibberellic acid, benzylaminopurine, abscisic acid, and ethrel	Foliar spray	5 µM	Withheld irrigation to induce permanent wilting point	Cotton (*Gossypium hirsutum* L.)	Foliar-applied BAP improved net photosynthetic rate, stomatal conductance, transpiration rate and lint mass per plant, while ABA improved seed number and lint mass per plant	Pandey *et al.* (2003)
Brassinolide	Foliar spray	0.1 mg L^{-1}	35% and 80% field capacity	Soybean (*Glycine max* L.)	Enhanced biomass accumulation, seed yield, chlorophyll content, photosynthetic rate, maximum quantum	Zhang *et al.* (2008)

					yield of PSII, activity of ribulose-1,5-bisphosphate carboxylase, leaf water potential, soluble sugars, proline, and the activities of peroxidase and superoxide dismutase	
Polyamines (Putrescine, spermidine, and spermine)	Seed priming and foliar spray	10 μM	50% of field capacity	Rice (*Oryza sativa* L.)	Improved net photosynthesis, water-use efficiency, leaf water status, free proline, anthocyanins, and soluble phenolics, and alleviated oxidative damage	Farooq *et al.* (2009)
Putrescine	Seed soaking	0.01 mM	0.2, 0.4, 0.6, 0.8 MPa	Alfalfa (*Medicago sativa* L.)	Decreased activities of glutamate-oxaloacetate transferase (GOT), glutamate-pyruvate transferase (GPT), and RNase and reduced total soluble nitrogenous compounds, while increased DNA, RNA, and protein contents	Zeid and Shedeed (2007)

assimilation rate, enhanced activities of some key antioxidant enzymes, and higher accumulation of photosynthetic pigments.

Exogenous application of polyamines also has been shown to be effective in mitigating harmful effects of drought stress in some plant species (Ashraf *et al.*, 2008; Saruhan *et al.*, 2006; Zeid and Shedeed, 2007). For example, foliar application of polyamines, putrescine, spermidine, or spermine overcame much of the drought-induced leaf rolling in *Ctenanthe setosa* L. (Saruhan *et al.*, 2006). Polyamines application led to decreased peroxidase activity and increased soluble proteins in leaves of the drought-stressed *C. setosa* plants. Further, in this study, while application of putrescine and spermidine caused increased accumulation of proline and reducing sugars, no such effects were observed with application of spermine (Saruhan *et al.*, 2006). Farooq *et al.* (2009) discovered that exogenous application of polyamines improved drought tolerance of rice plants, as measured by total dry mass and photosynthetic capacity under water stress. In this study, exogenous application of polyamines also resulted in improved photosynthetic capacity, leaf water content, WUE, and accumulation of free proline and some secondary metabolites (anthocyanins and soluble phenolics). This treatment also improved membrane integrity and upregulated some key antioxidant enzymes in drought-stressed rice plants.

From the above-mentioned studies, it is clear that exogenous application of PGRs may alleviate harmful effects of drought stress in at least some plant species. Further studies are needed to examine the usefulness of this approach in minimizing adverse effects of water deficit in major crop species. Also, further studies are needed to examine the mechanism underpinning such improvement. Currently, it is unknown whether beneficial effects of exogenous application of PGRs are via overcoming the imbalances in PGR concentrations normally caused by osmotic stress, or through other means. Further, the economy of using PGRs for improving plant stress tolerance must be taken into consideration. For example, some hormones such as BRs are very expensive, and thus the cost–benefit ratio may be too high to bear for their commercial use. For other hormones, this may not be an issue. Nonetheless, exogenous application of PGRs is a potential approach to consider for minimizing the adverse effects of drought stress in agriculture.

6. Role of Inorganic Nutrients in Plant Drought Tolerance

The availability of essential mineral nutrients in the soil is generally perturbed by drought stress, leading to nutritional deficiencies or imbalances in plants (Alam, 1999; Hu and Schmidhalter, 2005). Such drought-induced

impaired nutrition may occur by poor root growth and/or poor nutrient mobility in the soil (Fageria *et al.*, 2002; Samarah *et al.*, 2004). However, impaired nutrient availability in plants under drought stress may be caused by various factors, including interference in nutrient uptake and unloading mechanisms and decreased transpirational stream (Baligar *et al.*, 2001; Marschner, 1995).

There are varied and sometimes contrasting reports as to the role of nutrient supplementation in improving plant growth under drought stress. In general, under water-deficit conditions, the exogenous supply of nutrients may improve, reduce, or have no effects on plant growth, depending on the severity of drought, soil nutrient content, and other conditions (Alam, 1999; Hu and Schmidhalter, 2005). For example, when drought intensity is too severe or the soil is already nutrient rich, supplying additional nutrient would not help plant growth. However, when plants are provided with optimal amount of nutrients, they would require less water, compared to plants experiencing nutrient deficiency, because they exhibit a higher WUE (Hu and Schmidhalter, 2005). This is, in turn, because an adequate nutrient supply would promote root growth under drought conditions, which would provide for enhanced extraction of water and nutrient from deeper soil strata (Hu and Schmidhalter, 2005). A comprehensive knowledge of the roles of mineral nutrients in plant growth under drought stress will help improve fertilization management, particularly in area suffering from periods of drought stress. And as important is a good understanding of the effects of drought-stress on nutrient availability, absorption, transportation, accumulation, and partitioning in plants as well as potential interactions between nutrient application and plant response to drought stress.

7. Exogenous Application of Mineral Nutrients to Improve Drought Tolerance

7.1. Soil amendment with mineral nutrients

Several reports have shown that the damaging effects of drought-stress on plants could be reduced with proper supply of mineral nutrients (Ashraf *et al.*, 2001a,b; Khan *et al.*, 2004; Marschner, 1995; Payne *et al.*, 1995; Raun and Johnson, 1999; Sanchez-Bel *et al.*, 2008; Singh and Sale, 2000). For example, seed yield in wheat, corn, and barley increased in drought-prone lands with supply of additional nitrogen to the soil (Halvorson and Reule, 1994). Similarly, drought-induced growth reduction in maize (Li and Zheng, 1996), sorghum (*Sorghum bicolor* L.) (Sharma and Kumari, 1996), and mustard (*Brassica juncea* L.; Umar *et al.*, 1995) was alleviated by soil K supplementation. Also, soil application of P in wheat (Rodriguez *et al.*, 1996); N in wheat, maize, and barley (Halvorson and Reule, 1994); N, K,

and Ca in fig (*Ficus carica* L.; Irget *et al.*, 2008); and boron (B) in Norway spruce (*Picea abies* (L.) Karst) resulted in improved drought tolerance (Möttönen *et al.*, 2001). In Romaine lettuce (*Lactuca sativa* L.) grown under drought stress, soil fertilization with B reduced plant wilting, compared to plants which did not receive this treatment (Willis and Piland, 1937). Similarly, in B-deficient legumes grown under water stress, the decrease in transpiration rate was slower than plants which were provided with sufficient amount of B (Dorfmüller, 1941). Sharma and Sharma (1987) observed that leaves of B-deficient cauliflower (*B. oleracea* L.) had abnormal stomata and distorted guard cells and impaired growth under drought stress. Similarly, Sharma and Ramchandra (1990) reported that B-deficient mustard (*Brassica campestris* L.) plants grown under water-deficit conditions had relatively lower water potentials and exhibited decreased stomatal pore opening, reduced transpiration, and decreased intercellular CO_2 concentration, net photosynthesis, and dry matter yield, when compared to B-sufficient plants grown under the same drought conditions.

In summary, while among the diverse consequences of drought stress in plants, restriction in the uptake and accumulation of nutrients such as P, N, K^+, and Ca^{2+} is a common phenomenon, soil amendment with such nutrients may contribute to better plant growth and development under such conditions by facilitating further uptake of the nutrients. This observation can have significant implications in commercial production of crops under drought stress conditions.

7.2. Foliar application of mineral nutrients

The supply of mineral nutrients via the root medium is restricted under water-deficit conditions because of the adverse effects of drought on nutrient availability and uptake. Thus, foliar application of such nutrients has received considerable attention in the recent past, as this approach provides nutrients directly to the foliage and possibly it is a faster method of nutrient delivery, when compared to uptake from the soil. At early growth stages, foliar fertilization could increase nutrient supply at a time when the root system is not well developed. Foliar application of nutrients can also be very useful under field conditions where uptake of nutrients from the soil may be negatively affected by uncontrollable environmental factors. Thus, foliar application has been considered as an alternative means of nutrient delivery to the plant when plant nutrient deficiencies could not be remedied via soil amendment (Cakmak and Kirkby, 2008; Sarkar *et al.*, 2007). This method has been shown to improve plant growth under drought stress in several plant species. For example, foliar application of Zn, K, or Mg improved growth and seed yield of mungbean (*Vigna radiata* L.) plants grown under drought stress (Thalooth *et al.*, 2006). Among these minerals, the positive effects of K were more pronounced. Mazher *et al.* (2007) reported a

significant effect of foliar-applied K on improving growth of orchid tree (*Bauhinia variegata* L.) under drought stress. Further, foliar application of P was effective in improving growth, seed yield, WUE, and photosynthetic capacity in common beans grown under water-deficit conditions (dos Santos *et al.*, 2004).

In addition to macroelements, foliar application of trace elements has been shown to be effective in improving plant growth under drought stress in different plant species (Table 4). For example, foliar application of Zn and Mn on safflower (*Carthamus tinctorius* L.) plants grown under drought stress resulted in considerable improvement in vegetative growth, seed yield, and seed quality (Movahhedy-Dehnavy *et al.*, 2009). This is in agreement with the results of several earlier studies in which foliar application of Zn and Mn significantly improved growth of various plant species subjected to water-deficit conditions (Irget *et al.*, 2008; Mirzapour and Khoshgoftar, 2006; Thalooth *et al.*, 2006).

It should be noted that the efficiency of foliar application of nutrients is directly correlated with the mobility of the nutrient within the plant (Ashraf *et al.*, 2008; Mengel, 2002). For example, N, K, and Mg have fast mobility through the phloem, and thus their foliar applications would be highly beneficial in improving plant stress tolerance, whereas Ca and Fe have relatively slow mobility and thus they may be less effective (Marschner, 1995). Soil amendment of immobile nutrients can be very problematic, as their effectiveness depends on numerous soil factors (Papadakis *et al.*, 2007; Pestana *et al.*, 2001). An alternative and potentially more effective means to avoid nutrient deficiencies in plants is foliar application of ionic or chelated forms of nutrients, as discussed elsewhere (Ashraf and Foolad, 2005; Marschner, 1995). For example, in citrus plants, the effectiveness of foliar application of $MnSO_4$ was found to be higher as compared to that of soil application (Papadakis *et al.*, 2005). Similarly, foliar application of Fe in the form of $FeSO_4$ to citrus trees was found relatively more effective than its soil application (Papadakis *et al.*, 2007).

7.3. Presowing seed treatment with mineral nutrients

Mineral nutrients can also be applied as a presowing seed treatment. Such a treatment, known as halopriming (Ashraf and Foolad, 2005; Kaur *et al.*, 2002), can improve not only the rate and uniformity of seed germination but also plant growth under drought stress (Casenave and Toselli, 2007; Iqbal and Ashraf, 2005; Patade *et al.*, 2009). Salim and Todd (1968) reported that wheat and barley kernels haloprimed with $CaCl_2$ solution exhibited improved desiccation tolerance in terms of seed germination, plant growth, and transpiration. Seed priming has been reported to stimulate vegetative growth and final yield in many crop plants, including chickpea (Kaur *et al.*, 2002), cotton (Casenave and Toselli, 2007), and wheat (Iqbal and Ashraf,

Table 4 Regulation of various physiological and biochemical processes in different plant species by exogenous application of inorganic nutrients under drought stress

Mineral nutrients	Mode of application	Concentration applied	Level of drought stress used	Species	Regulation in growth and physiobiochemical processes	Reference
Nitrogen	Soil application	224, 336, and 448 mg kg^{-1}	30% field capacity	Pearl millet (*Pennisetum glaucum* L.)	Improved growth, net assimilation rate, transpiration rate, leaf turgor potential, and relative growth rate. Increased shoot and root N, P, K, and Ca contents	Ashraf *et al.* (2001a,b)
	Soil application	2, 6, 10, and 20 g m^{-2}	30% field capacity	Creeping bentgrass (*Agrostis palustris* Huds.)	Enhanced cell membrane stability, leaf turgor, nutrient contents (N, K, Ca) and glycinebetaine levels, and prevented cell membrane damage	Saneoka *et al.* (2004)
Nitrogen and phosphate	Soil application	49, 123.6, 165.0, 206.4, and 281.0 kg ha^{-1} of N (urea) and 44.4, 75, 105.6, and 150 kg ha^{-1} P (lime superphosphate)	43%, 54%, 60%, 66%, and 77% field capacities	Maize (*Zea mays* L.)	Improved plant growth, grain yield, and water-use efficiency	Zhan-Xiang *et al.* (2009)
Potassium	Foliar spray	0.25 and 50 mg L^{-1}	Irrigation interval 3, 4, 5, and 6 day	Butterfly tree (*Bauhinia variegate* L.)	Enhanced root growth, chlorophyll content, sugar, and uptake and accumulation of N, P, and K in all plant organs at 50 mg L^{-1}	Mazher *et al.* (2007)
	Soil application	235, 352.5, and 470 mg kg^{-1}	30% field capacity	Pearl millet	Improved shoot and root N and K contents	Ashraf *et al.* (2002)

	Through rooting medium	2.5 and 10 mM as K_2SO_4	−1.5 MPa leaf water potential	Hibiscus (*Hibiscus rosa-sinensis* L.)	Improved root longevity, photosynthesis, transpiration, stomatal conductance, and leaf water content (LWC), while decreased leaf osmotic potential	Egilla *et al*. (2001, 2005)
	Soil application	50, 100, and 200 mg/pot	−15 bars	Maize	Improved leaf area expansion, leaf area per plant, stomatal conductance, and nitrate reductase activity	Khanna-Chopra *et al*. (2006)
	Soil application	0, 30, 60, and 120 mg kg^{-1} soil in pots and 25, 50, and 75 kg ha^{-1} in field	Drought imposed by withholding water	Mustard (*Brassica juncea* L.), sorghum (*Sorghum bicolor* L. Moench), and groundnut (*Arachis hypogaea* L.)	Improved plant biomass, grain yield, leaf K^+ content, and relative water content (RWC)	Umar (2006)
Potassium humate	Foliar spray	250 mL ha^{-1}	30 and 60 mm precipitation	Potato (*Solanum tuberosum* L.)	Improved tuber yield (0.93–9.63 t ha^{-1}), plant height, tuber number, and weight per plant	Hassanpanah (2009)
Phosphorus and thiourea	Presowing seed treatment + foliar spray	500 and 1000 μg g^{-1} thiourea and 40 kg P ha^{-1}	Water stress maintained by irrigation at 8 day interval	Clusterbean (*Cyamopsis tetragonoloba* Taub.)	Enhanced net photosynthesis, leaf area, chlorophyll content, and nitrogen metabolism leading to a significant improvement in plant growth and seed yield under water-stress conditions	Burman *et al*. (2004)
Phosphorus	Foliar spray	10 and 20 g L^{-1}	−0.9 MPa leaf water potential	Common bean (*Phaseolus vulgaris* L.)	Improved seed dry weight, photosynthetic rate, and intrinsic water-use efficiency	dos Santos *et al*. (2004)

(*Continued*)

Table 4 (*Continued*)

Mineral nutrients	Mode of application	Concentration applied	Level of drought stress used	Species	Regulation in growth and physiobiochemical processes	Reference
	Foliar spray	10 g L^{-1}	−1.1 MPa leaf water potential	Common bean	Enhanced photosynthetic rate, stomatal conductance, O_2 evolution (Ac), and nonphotochemical quenching (NPQ)	dos Santos *et al.* (2006)
	Soil application	20 and 80 mg kg^{-1}	60% and 35% of the 10 kPa soil–water content	Soybean (*Glycine max* L. (Merr.)	Improved number of lateral branches and plant development, while leaf area development was not affected	Flavio *et al.* (2001)
Zinc (Zn) and manganese (Mn)	Foliar spray	3000 mg L^{-1}	Withheld irrigation during vegetative, flowering, and seed filling stages	Safflower (*Carthamus tinctorius* L.)	Enhanced germination rate, germination percentage, seedling dry weight, final seedling emergence, Zn and Mn contents, proteins, and major proteins fractions, and oil fatty acid contents (linoleic acid and oleic acid)	Movahhedy-Dehnavy *et al.* (2009)
Zinc, potassium, and magnesium	Foliar spray	300 mg L^{-1} Zn–EDTA, 2.0% KNO_3, and 50 mg L^{-1} $MgSO_4$	Dropping/eliminating one irrigation at each of vegetative, flowering, and pod formation growth stages	Mungbean (*Vigna radiata* L. Wilczek)	Zn, K, or Mg application improved area, number, and weight of leaves as well as number and weight of pods per plant, plant height, number of branches per plant, and stem dry weight	Thalooth *et al.* (2006)

Zinc	Soil application	20 mg L^{-1}	Four regimes of soil matric potential (Ψ_m) −0.03, −0.5, −0.1, and −0.15 MPa	Soybean	Zn improved shoot and root growth at all levels of stress	Gadallah (2000)
Thiourea	Presowing seed treatment + foliar spray	500 and 1000 mg L^{-1}	Rainfed area at 174 mm soil precipitation	Mungbean	Improved dry matter production, pod number, pod weight, seed yield, photosynthetic rate, total chlorophyll, starch, reducing sugars, soluble proteins, and nitrate reductase activity	Mathur *et al.* (2006)
Selenium	Foliar spray	20 g ha^{-1}	15% moisture contents	Maize	Improved 1000 grain weight, grain yield, harvest index, and water-use efficiency	Sajedi *et al.* (2009)

2007). Further, it has been reported that seedlings developed from primed seeds generally emerge faster and grow more vigorously (Iqbal and Ashraf, 2005; Shad *et al.*, 2001). Yagmur and Kaydan (2008) reported that seed of hexaploid triticale (*X. Triticosecale* Witmack cv. Presto) pretreated with KH_2PO_4 solution showed improved germination and seedling growth under osmotic stress caused by NaCl or PEG. Similarly, achenes of sunflower pretreated with KNO_3 solution exhibited enhanced germination and seedling growth under osmotic stress (Kaya *et al.*, 2006), and saplings of mangrove (*Bruguiera cylindrica* L.) raised from propagules pretreated with NaCl solution showed enhanced desiccation tolerance (Atreya *et al.*, 2009). In conclusion, it appears that similar to foliar application, the presowing seed treatment with solutions of various inorganic salts is a promising approach to alleviating the damaging effects of drought stress during both early and late stages of plant growth and development. However, further research is needed to determine the optimal dose of each salt and the time period for soaking the seed in the salt solution.

8. Conclusions and Future Prospects

Organic osmolytes, PGRs, and mineral nutrients play essential roles in modulating plant growth and development under stress and nonstress conditions. In general, plants often perform better when intracellular levels of these substances are optimal. In contrast, plants that cannot naturally accumulate sufficient amount of organic osmolytes, and PGRs generally have reduced growth under stress conditions. However, such plants can be tailored to grow better in stressful environments either by increasing the intrinsic levels of these compounds through genetic modifications or by exogenous application of the corresponding compounds. Although some progress has been made in developing transgenic plants with enhanced endogenous production of osmolytes and PGRs, in many cases the intrinsic levels of these compounds have not been high enough to improve plant stress tolerance. Currently, efforts are underway to develop transgenic plants capable of producing higher levels of such compounds. However, recently a great attention has been devoted to enhancing plant performance under stress conditions by exogenous application of organic osmolytes, PGRs, or mineral nutrients via presowing seed treatment, foliar application, or soil amendment.

Most crop plants experience reduced productivity under drought stress. It is generally agreed that in most cases, poor crop stand establishment at the beginning of the growing season is the major cause of such reduced productivity. It is also generally agreed that rapid and uniform seed germination and seedling emergence have direct correlation with good crop stand

establishment and higher crop yield. Further, it is known that vigorous plants with well-developed root systems are more resistant to stresses than plants with poor vigor or whose growth and development have been hampered during early development. As discussed in this review, an effective approach to improving plant performance under drought stress is through improving germination and stand establishment via presowing seed treatment with organic osmolytes, PGRs, or certain mineral nutrients. There are many noted examples where priming seed with such substances has resulted in improved drought tolerance in different plant species. Organic osmolytes, PGRs, and mineral nutrients could also be applied via foliar spray, and in case of mineral nutrients also via soil amendment. The exogenous application strategy to improve plant stress tolerance has gained considerable attention because of its general efficiency, feasibility, and cost- and labor-effectiveness. However, undoubtedly, not all plant species respond similarly to such treatments, and there are variations among plants in terms of the substance to use, required concentration, or treatment period. Thus, for each plant species, the most useful substance(s) and optimal treatment conditions should be determined in advance.

Organic osmolytes and PGRs are normally produced in specific concentrations in different plant species to maintain optimal growth. However, their concentrations and ratios may be disturbed in response to stress, which may constitute a major factor causing poor plant growth and development under water-limited conditions. Yet this disturbance in stressed plants can potentially be remedied by exogenous application of the desired organic osmolytes or PGRs, and thus facilitating a normal growth and production. However, exogenous application of a particular organic osmolyte or PGR may suppress the inherent ability of a plant to synthesize this compound, or perturb the optimal ratios of other PGRs or organic osmolytes. Thus, for effective use of this strategy, it is important to have a thorough knowledge of the regulation of the intrinsic levels of different osmolytes and PGRs and their potential interactions in the plant. In some cases, exogenous application of certain osmolytes or PGRs may not help enhancing plant drought tolerance. This may not be a concern with mineral nutrients, for which their exogenous application would not interfere with other essential compounds inside the plants such as organic osmolytes and PGRs. However, exogenous application of mineral nutrients also would not always improve plant stress tolerance. For example, if the water-limited soil is already rich in nutrients or the intensity of the drought is too high, such treatments would not be beneficial. In fact, under such conditions, exogenous application of nutrients may have negative effects on the plant. Therefore, before considering exogenous application of organic osmolytes, PGRs, or mineral nutrients to improve plant drought tolerance under large-scale commercial planting, effects of other influencing factors should be determined.

An important consideration in the use of growth-promoting factors to improve plant stress tolerance is the cost–benefit ratio. In the case of some compounds, such as osmolytes, trehalose, and proline, and PGR brassinosteroids, this ratio is currently too high, prohibiting their economic use in large scale. However, for use in small amount, such as in the case of presowing seed treatment, their application may be economically feasible. As for foliar application, normally large quantities of PGRs or organic osmolytes are required. In such situations, alternative and cheaper sources of such compounds should be identified. One example is synthetic GB, which is quite expensive. However, some plants such as sugar beet and barley have been identified which are naturally rich sources of GB. For example, the concentration of GB in sugar beet ranges from 50 to 100 mM, and it has been suggested that simple sugar beet extract, which can be obtained inexpensively, could be used for presowing seed treatment or foliar spray to improve plant stress tolerance. However, further research is needed to identify alternative and cheaper sources of other important organic osmolytes or PGRs to facilitate their economic use in large scale to enhance plant stress tolerance.

ACKNOWLEDGMENTS

We thank Dr. Rob Briddon, Visiting Professor, National Institute of Biotechnology and Genetic Engineering, Faisalabad, Pakistan, for critically reading the chapter and providing useful suggestions.

REFERENCES

Abebe, T., Guenzi, A. C., Martin, B., and Cushman, J. C. (2003). Tolerance of mannitol-accumulating transgenic wheat to water stress and salinity. *Plant Physiol.* **131,** 1748–1755.

Abreu, M. E., and Munne-Bosch, S. (2008). Salicylic acid may be involved in the regulation of drought-induced leaf senescence in perennials: A case study in field-grown *Salvia officinalis* L. plants. *Environ. Exp. Bot.* **64,** 105–112.

Agboma, M., Jones, M. G. K., Peltonen-Sainio, P., Rita, H., and Pehu, E. (1997). Exogenous glycinebetaine enhances grain yield of maize, sorghum and wheat grown under two supplementary watering regimes. *J. Agron. Crop Sci.* **178,** 29–37.

Aharoni, N., Blumenfeld, A., and Richmond, A. E. (1977). Hormonal activity detached lettuce leaves as affected by leaf water content. *Plant Physiol.* **59,** 1169–1173.

Ahmadi, A., Sio-Se, A. M., Poustini, K., and Jahromi, M. (2007). Influence of osmo and hydropriming on seed germination and seedling growth in wheat (*Triticum aestivum* L.) cultivars under different moisture and temperature conditions. *Pak. J. Biol. Sci.* **10,** 4043–4049.

Akashi, K., Miyake, C., and Yokota, A. (2001). Citrulline, a novel compatible solute in drought-tolerant wild watermelon leaves, is an efficient hydroxyl radical scavenger. *FEBS Lett.* **508,** 438–442.

Akram, N. A., Shahbaz, M., and Ashraf, M. (2007). Relationship of photosynthetic capacity and proline accumulation with the growth of differently adapted populations of two potential grasses (*Cynodon dactylon* (L.) Pers. and *Cenchrus ciliaris* L.) to drought stress. *Pak. J. Bot.* **39,** 777–786.

Akram, N. A., Shahbaz, M., and Ashraf, M. (2008). Nutrient acquisition in differentially adapted populations of *Cynodon dactylon* (L.) Pers. and *Cenchrus ciliaris* L. under drought stress. *Pak. J. Bot.* **40,** 1433–1440.

Akula, A., Becker, D., and Bateson, M. (2000). High-yielding repetitive somatic embryogenesis and plant recovery in a selected tea clone, 'TRI-2025', by temporary immersion. *Plant Cell Rep.* **19,** 1140–1145.

Alam, S. M. (1999). Nutrient uptake by plants under stress conditions. *In* "Plant and Crop Stress" (M. Pessarakli, Ed.), pp. 285–313. Marcel Dekker, NY, USA.

Ali, Q., Ashraf, M., and Athar, H. R. (2007). Exogenously applied proline at different growth stages enhances growth of two maize cultivars grown under water deficit conditions. *Pak. J. Bot.* **39,** 1133–1144.

Ali, Q., Ashraf, M., Shahbaz, M., and Humera, H. (2008). Ameliorating effect of foliar applied proline on nutrient uptake in water stressed maize (*Zea mays* L.) plants. *Pak. J. Bot.* **40,** 211–219.

Allard, F., Houde, M., Krol, M., Ivanov, A., Huner, N. P. A., and Sarhan, F. (1998). Betaine improves freezing tolerance in wheat. *Plant Cell Physiol.* **39,** 1194–1202.

Alscher, R. G., Erturk, N., and Heath, L. S. (2002). Role of superoxide dismutase (SODs) in controlling oxidative stress in plants. *J. Exp. Bot.* **53,** 133–141.

Alvarez, I., Tomaro, M. L., and Benavides, M. P. (2003). Changes in polyamines, proline and ethylene in sunflower calluses treated with NaCl. *Plant Cell Tissue Organ Cult.* **74,** 51–59.

Amin, B., Mahleghah, G., Mahmood, H. M. R., and Hossein, M. (2009). Evaluation of interaction effect of drought stress with ascorbate and salicylic acid on some physiological and biochemical parameters in Okra (*Hibiscus esculentus* L.). *Res. J. Biol. Sci.* **4,** 380–387.

Ashraf, M. (2009). Biotechnological approach of improving plant salt tolerance using antioxidants as markers. *Biotechnol. Adv.* **27,** 84–93.

Ashraf, M. (2010). Inducing drought tolerance in plants: Recent advances. *Biotechnol. Adv.* **28,** 169–183.

Ashraf, M., and Foolad, M. R. (2005). Pre-sowing seed treatment-a shotgun approach to improve germination, plant growth, and crop yield under saline and non-saline conditions. *Adv. Agron.* **88,** 223–271.

Ashraf, M., and Foolad, M. R. (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environ. Exp. Bot.* **59,** 206–216.

Ashraf, M., and Harris, P. J. C. (2004). Potential biochemical indicators of salinity tolerance in plants. *Plant Sci.* **166,** 3–16.

Ashraf, M., Shahbaz, M., and Ashraf, M. Y. (2001a). Influence of nitrogen supply and water stress on growth and nitrogen, phosphorus, potassium and calcium contents in pearl millet. *Biol. Plant.* **44,** 459–462.

Ashraf, M., Shahbaz, M., Mahmood, S., and Rasul, E. (2001b). Relationships between growth and photosynthetic characteristics in pearl millet (*Pennzsetum glaucum*) under limited water deficit conditions with enhanced nitrogen supplies. *Belg. J. Bot.* **134,** 1–14.

Ashraf, M., Ashfaq, M., and Ashraf, M. Y. (2002). Effects of increased supply of potassium on growth and nutrient content in pearl millet under water stress. *Biol. Plant.* **45,** 141–144.

Ashraf, M., Ali, Q., and Rha, E. S. (2005). The effect of applied nitrogen on the growth and nutrient concentration of Kalonji (*Nigella sativa*). *Aust. J. Exp. Agric.* **45,** 459–463.

Ashraf, M., Athar, H. R., Harris, P. J. C., and Kwon, T. R. (2008). Some prospective strategies for improving crop salt tolerance. *Adv. Agron.* **97,** 45–110.

Athar, H. R., and Ashraf, M. (2009). Strategies for crop improvement against salinity and drought stress: An overview. *In* "Salinity and Water Stress Improving Crop Efficiency" (M. Ashraf, M. Ozturk, and H. R. Athar, Eds.), Task for Vegetation Science, Vol. 44, pp. 1–16.

Atreya, A., Vartak, V., and Bhargava, S. (2009). Salt priming improves tolerance to desiccation stress and extreme salt stress in *Bruguiera cylindrical*. *Int. J. Integr. Biol.* **6,** 68–73.

Avonce, N., Leyman, B., Mascorro-Gallardo, J. O., Van Dijck, P., Thevelein, J., and Iturriaga, G. (2004). The *Arabidopsis trehalose-6P-synthase AtTPS1* gene is a regulator of glucose, ABA and stress signaling. *Plant Physiol.* **136,** 3649–3659.

Ayub, N., Bano, A., Ramzan, S., and Usman, M. (2000). Effect of VAM on drought tolerance and growth of plant in comparison with the effect of growth regulators. *Pak. J. Biol. Sci.* **3,** 957–959.

Bajguz, A., and Hayat, S. (2009). Effects of brassinosteroids on the plant responses to environmental stresses. *Plant Physiol. Biochem.* **47,** 1–8.

Baligar, V. C., Fageria, N. K., and He, Z. L. (2001). Nutrient use efficiency in plants. *Commun. Soil Sci. Plant Anal.* **32,** 921–950.

Bernier, J., Kumar, A., Venuprasad, R., Spaner, D., Verulkar, S., Mandal, N. P., Sinha, P. K., Peeraju, P., Dongre, P. R., Mahto, R. N., and Atlin, G. (2009). Characterization of the effect of a QTL for drought resistance in rice, *qtl12.1*, over a range of environments in the Philippines and eastern India. *Euphytica* **166,** 207–217.

Berova, M., and Zlatev, Z. (2000). Physiological response and yield of paclobutrazol treated tomato plants (*Lycopersicon esculentum* Mill.). *Plant Growth Regul.* **30,** 117–123.

Blanchard, M. G., Newton, L. A., Runkle, E. S., Woolard, D., and Campbell, C. A. (2007). Exogenous applications of abscisic acid improved the postharvest drought tolerance of several annual bedding plants. *Acta Hort.* **55,** 127–132.

Blum, A. (2005). Drought resistance, water-use efficiency and yield potential are they compatible, dissonant or mutually exclusive? *Aust. J. Agric. Res.* **56,** 1159–1168.

Blum, A. (2009). Effective use of water (EUW) and water-use efficiency (WUE) is the target of crop yield improvement under drought stress. *Field Crops Res.* **112,** 119–123.

Bohnert, H. J., and Shen, B. (1999). Transformation and compatible solutes. *Sci. Hort.* **78,** 237–260.

Bray, E. A. (1997). Plant responses to water deficit. *Trends Plant Sci.* **2,** 48–54.

Bray, E. A., Bailey-Serres, J., and Weretilnyk, E. (2000). Responses to abiotic stresses. *In* "Biochemistry and Molecular Biology of Plants" (W. Gruissem, B. Buchannan, and R. Jones, Eds.), pp. 158–249. American Society of Plant Physiologists, Rockville, MD.

Burger, A. (2009). Drought a growing threat to world agriculture, food production; http://globalwarmingisreal.com/2009/02/26/drought-a-growing-threat-to-world-agriculture-food-production/.

Burman, U., Garg, B. K., and Kathju, S. (2004). Interactive effects of thiourea and phosphorus on clusterbean under water stress. *Biol. Plant.* **48,** 61–65.

Cakmak, I., and Kirkby, E. A. (2008). Role of magnesium in carbon partitioning and alleviating photooxidative damage. *Physiol. Plant.* **133,** 692–704.

Cano-Delgado, A., Yin, Y., Yu, C., Vafeados, D., Mora-Garcia, S., Cheng, J. C., Nam, K. H., Li, J., and Chory, J. (2004). BRL1 and BRL3 are novel brassinosteroid receptors that function in vascular differentiation in *Arabidopsis*. *Development* **131,** 5341–5351.

Cao, H. X., Zhang, Z. B., Xu, P., Chu, L. Y., Shao, H. B., Lu, Z. H., and Liu, J. H. (2007). Mutual physiological genetic mechanism of plant high water use efficiency and nutrition use efficiency. *Colloids Surf., B* **57,** 1–7.

Capell, T., Bassie, L., and Christou, P. (2004). Modulation of the polyamine biosynthetic pathway in transgenic rice confers tolerance to drought stress. *Proc. Natl. Acad. Sci. USA* **101,** 9909–9914.

Casenave, E. C., and Toselli, M. E. (2007). Hydropriming as a pre-treatment for cotton germination under thermal and water stress conditions. *Seed Sci. Technol.* **35,** 88–98.

Catala, R., Ouyang, J., Abreu, I. A., Hu, Y., Seo, H., Zhang, X., and Chua, N. H. (2007). The *Arabidopsis* E3 sumo ligase SIZ1 regulates plant growth and drought responses. *Plant Cell* **19,** 2952–2966.

Chaney, W. R., Premachandra, G. S., and Holt, H. A. (1996). Physiological basis for benefits of tree growth regulators. *In* "Proceedings, 8th Annual Conference Western Plant Growth Regulator Society, Sacramento, California," pp. 8–18.

Chaves, M. M., Maroco, J. P., and Pereira, J. S. (2003). Understanding plant response to drought: From genes to the whole plant. *Funct. Plant Biol.* **30,** 239–264.

Chen, T. H. H., and Murata, N. (2002). Enhancement of tolerance to abiotic stress by metabolic engineering of betaines and other compatible solutes. *Curr. Opin. Plant Biol.* **5,** 250–257.

Chernyadev, I. I. (2005). Effect of water stress on the photosynthetic apparatus of plants and the protective role of cytokinins: A review. *Appl. Biochem. Microbiol.* **41,** 115–128.

Chiappetta, A., Michelotti, V., Fambrini, M., Bruno, L., Salvini, M., Petrarulo, M., Azmi, A., Van Onckelen, H., Pugliesi, C., and Bitonti, M. B. (2006). Zeatin accumulation and misexpression of a class I *knox* gene are intimately linked in the epiphyllous response of the interspecific hybrid EMB-2 (*Helianthus annuus* × *Helianthus tuberosus*). *Planta* **223,** 917–931.

Chimenti, C. A., Marcantonio, M., and Hall, A. J. (2006). Divergent selection for osmotic adjustment results in improved drought tolerance in maize (*Zea mays* L.) in both early growth and flowering phases. *Field Crops Res.* **95,** 305–315.

Creelman, R. A., and Mullet, J. E. (1997). Biosynthesis and action of jasmonates in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **48,** 355–381.

Cushman, J. C. (2001). Osmoregulation in Plants: Implications for agriculture. *Am. Zool.* **41,** 758–769.

Davies, P. J. (2004). The plant hormones: Their nature, occurrence and function. *In* "Plant Hormones, Biosynthesis, Signal Transduction, Action" (P. J. Davies, Ed.). Kluwer, Dordrecht.

Davies, W. J., Metcalfe, J., Lodge, T. A., and Rosa de Costa, A. (1986). Plant growth substances and the regulation of growth under drought. *Aust. J Plant Physiol.* **13,** 105–125.

Davies, W. J., and Zhang, J. (1991). Root signals and the regulation of growth and development of plants in drying soil. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **42,** 55–76.

de-Carbonnel, E. (2009). Catastrophic Fall in 2009, Global Food Production. Global Res. E-Newsletter, www.globalresearch.ca/index.php?context=va&aid=12252.

Denby, K., and Gehring, C. (2005). Engineering drought and salinity tolerance in plants: Lessons from genome-wide expression profiling in *Arabidopsis*. *Trends Biotechnol.* **23,** 547–552.

Djilianov, D., Georgieva, T., Moyankova, D., Atanassov, A., Shinozaki, K., Smeeken, S. C. M., Verma, D. P. S., and Murata, N. (2005). Improved abiotic stress tolerance in plants by accumulation of osmoprotectants—Gene transfer approach. *Biotechnol. Biotechnol. Eq.* **19,** 63–71.

Dorfmüller, W. (1941). Über den einfuss des Bor auf den Wasserhaushalt der Leguminosen. *Planta* **32,** 51–66.

dos Santos, M. G., Ribeiro, R. V., de Oliveira, R. F., and Pimente, C. (2004). Gas exchange and yield response to foliar phosphorus application in *Phaseolus vulgaris* L. under drought. *Braz. J. Plant Physiol.* **16,** 171–179.

dos Santos, M. G., Ribeiro, R. V., de Oliveira, R. F., Machado, E. C., and Pimentel, C. (2006). The role of inorganic phosphate on photosynthesis recovery of common bean after a mild water deficit. *Plant Sci.* **170,** 659–664.

Egilla, J. N., Davies, F. T., and Drew, C. M. (2001). Effect of potassium on drought resistance of *Hibiscus rosa-sinensis* cv. Leprechaun: Plant growth, leaf macro- and micronutrient content and root longevity. *Plant Soil.* **229,** 213–224.

Egilla, J. N., Davies, F. T., and Boutton, T. W. (2005). Drought stress influences leaf water content, photosynthesis and water-use efficiency of *Hibiscus rosa-sinensis* at three potassium concentrations. *Photosynthetica* **43,** 135–140.

Elbein, A. D., Pan, Y. T., Pastuszak, I., and Carroll, D. (2003). New insight on trehalose: A multifunctional molecule. *Glycobiology* **13,** 17–27.

Elkoca, E., Kantar, F., and Fiahin, F. (2008). Influence of nitrogen fixing and phosphorus solubilizing bacteria on the nodulation, plant growth, and yield of chickpea. *J. Plant Nutr.* **31,** 157–171.

Fageria, N. K., Baligar, V. C., and Clark, R. B. (2002). Micronutrients in crop production. *Adv. Agron.* **77,** 185–268.

Farooq, M., Basra, S. M. A., Wahid, A., Cheema, Z. A., Cheema, M. A., and Khaliq, A. (2008). Physiological role of exogenously applied glycinebetaine to improve drought tolerance in fine grain aromatic rice (*Oryza sativa* L.). *J. Agron. Crop Sci.* **194,** 325–333.

Farooq, M., Wahid, A., and Lee, D. J. (2009). Exogenously applied polyamines increase drought tolerance of rice by improving leaf water status, photosynthesis and membrane properties. *Acta Physiol. Plant.* **31,** 937–945.

Fernández, J. A., Balenzategui, L., Bañón, S., and Franco, J. A. (2006). Induction of drought tolerance by paclobutrazol and irrigation deficit in *Phillyrea angustifolia* during the nursery period. *Sci. Hort.* **107,** 277–283.

Flavio, H., Boem, G., and Thomas, W. G. (2001). Leaf area development in soybean as affected by phosphorus nutrition and water deficit. *J. Plant Nutr.* **24,** 1711–1729.

Flexas, J., Bota, J., and Cifre, J. (2004). Understanding down regulation of photosynthesis under water stress, future prospects and searching for physiological tools for irrigation management. *Ann. Appl. Biol.* **144,** 273–283.

Fonseca, S., Chini, A., Hamberg, M., Adie, B., Porzel, A., Kramell, R., Miersch, O., Wasternack, C., and Solano, R. (2009). (+)-7-iso-Jasmonoyl-L-isoleucine is the endogenous bioactive jasmonate. *Nat. Chem. Biol.* **5,** 344–350.

Gadallah, M. A. A. (1995). Effect of water stress, abscisic acid and proline on cotton plants. *J. Arid Environ.* **30,** 315–325.

Gadallah, M. A. A. (2000). Effects of indole-3-acetic acid and zinc on the growth, osmotic potential and soluble carbon and nitrogen components of soybean plants growing under water deficit. *J. Arid Environ.* **44,** 451–467.

Galston, A. W. (2001). Plant biology: Retrospect and prospect. *Curr. Sci.* **80,** 143–152.

Garg, A. K., Kim, J. K., Owens, T. G., Ranwala, A. P., Choi, Y. D., Kochian, L. V., and Wu, R. J. (2002). Trehalose accumulation in rice plants confers high tolerance levels to different abiotic stresses. *Proc. Natl. Acad. Sci. USA* **99,** 15898–15903.

Geerts, S., and Raes, D. (2009). Deficit irrigation as an on-farm strategy to maximize crop water productivity in dry areas. *Agric. Water Manage.* **96,** 1275–1284.

Gemperlova, L., Nováková, M., Vaňková, R., Eder, J., and Cvikrová, M. (2006). Diurnal changes in polyamine content, arginine and ornithine decarboxylase, and diamine oxidase in tobacco leaves. *J. Exp. Bot.* **57,** 1413–1421.

Ghana, S. G., and William, F. S. (2003). Seed priming winter wheat for germination, emergence and yield. *Crop Sci.* **43,** 2135–2141.

Ghassemian, M., Nambara, E., Cutler, S., Kawaide, H., Kamiya, Y., and McCourt, P. (2000). Regulation of abscisic acid signaling by the ethylene response pathway in *Arabidopsis*. *Plant Cell* **12,** 1117–1126.

Giulivo, C., Ponchia, G., Gianola, A., and Pitacco, A. (1985). Effect of rootstock on water balance of golden delicious apple trees. *Acta Hort.* **171,** 399–404.

Goicoechea, N., Antolín, M. C., and Sánchez-Díaz, M. (1997). Gas exchange is related to the hormonal balance in mycorrhizal or nitrogen-fixing alfalfa subjected to drought. *Physiol. Plant.* **100,** 989–997.

Gomes, F. P., Oliva, M. A., Mielke, M. S., de Almeida, A. A. F., Leite, H. G., and Aquino, L. A. (2008). Photosynthetic limitations in leaves of young Brazilian green dwarf coconut (*Cocos nucifera* L. 'nana') palm under well-watered conditions or recovering from drought stress. *Environ. Exp. Bot.* **62,** 195–204.

Griffin, J. J., Thomas, G. R., and Mason, P. D. (2004). Heat and drought influence photosynthesis, water relations, and soluble carbohydrates of two ecotypes of redbud (*Cercis canadensis*). *J. Am. Hort. Sci.* **129,** 497–502.

Grossi, J. A. S., de Moraes, P. J., de Araújo Tinoco, S., Barbosa, J. G., and Finger, F. L. (2005). Effects of paclobutrazol on growth and fruiting characteristics of Pitanga ornamental pepper. *Acta Hort.* **683,** 333–336.

Grossmann, K. (2003). Mediation of herbicide effects by hormone interactions. *J. Plant Growth Regul.* **2,** 109–122.

Guan, Y., Hu, J., Wang, X., and Shao, C. (2009). Seed priming with chitosan improves maize germination and seedling growth in relation to physiological changes under low temperature stress.MSc. thesis *J. Zhejiang Univ. Sci.* **10,** 427–433.

Guinn, G., and Brummett, D. L. (1988). Changes in free and conjugated indole-3-acetic acid and abscisic acid in young cotton fruits and their abscission zones in relation to fruit retention during and after moisture stress. *Plant Physiol.* **86,** 28–31.

Guo, X. Y., Zhang, X. S., and Huang, Z. Y. (2010). Drought tolerance in three hybrid poplar clones submitted to different watering regimes. *J. Plant Ecol.* **3,** 79–87.

Haberer, G., and Keiber, J. J. (2002). Cytokinins, new insight into a classic phytohormone. *Plant Physiol.* **128,** 354–362.

Hajihashemi, S., Kiarostami, K., Enteshari, S., and Saboora, A. (2009). Effect of paclobutrazol on wheat salt tolerance at pollination stage. *Russ. J. Plant Physiol.* **56,** 251–257.

Halvorson, A. D., and Reule, C. A. (1994). Nitrogen fertilizer requirements in an annual dryland cropping system. *Agron. J.* **86,** 315–318.

Hamilton, E. M., and Heckathorn, S. A. (2001). Mitochondrial adaptations to NaCl complex I is protected by anti-oxidants and small heat shock proteins, whereas complex II is protected by proline and betaine. *Plant Physiol.* **126,** 1266–1274.

Han, S. E., Park, S. R., Kwon, H. B., Yi, B. Y., Lee, G. B., and Byun, M. O. (2005). Genetic engineering of drought-resistant tobacco plants by introducing the trehalose phosphorylase (TP) gene from *Pleurotus sajor-caju*. *Plant Cell Tissue Organ Cult.* **82,** 151–158.

Hanson, A. D., and Scott, N. A. (1980). Betaine synthesis from radioactive precursors in attached, water-stressed barley leaves. *Plant Physiol.* **66,** 342–348.

Harinasut, P., Tsutsui, K., Takabe, T., Nomura, M., Takabe, T., and Kishitani, S. (1996). Exogenous glycinebetaine accumulation and increased salt-tolerance in rice seedlings. *Biosci. Biotechnol. Biochem.* **60,** 366–368.

Harris, D. (1996). The effects of manure, genotype, seed priming, depth and date of sowing on the emergence and early growth of *Sorghum bicolor* (L.) Moench in semi-arid Botswana. *Soil Tillage Res.* **40,** 73–88.

Harris, D., Joshi, A., Khan, P. A., Gothkar, P., and Sodhi, P. S. (1999). On-farm seed priming in semi-arid agriculture: Development and evaluation in maize, rice and chickpea in India using participatory methods. *Exp. Agric.* **35,** 15–29.

Harris, D., Raghuwanshi, B. S., Gangwar, J. S., Singh, S. C., Joshi, K. D., Rashid, A., and Hollington, P. A. (2001). Participatory evaluation by farmers of 'on-farm' seed priming in wheat in India, Nepal and Pakistan. *Exp. Agric.* **37,** 403–415.

Harris, D., Tripathi, R. S., and Joshi, A. (2002). On-farm seed priming to improve crop establishment and yield in dry direct-seeded rice. *In* "Proceedings of the International Workshop on Direct Seeding in Asian Rice Systems: Strategic Research Issues and

Opportunities" (S. Pandey, M. Mortimer, L. Wade, T. P. Tuong, K. Lopez, and B. Hardy, Eds.), pp. 231–240. International Rice Research Institute, Los Banos, Philippines.

Hassanpanah, D. (2009). Effects of water deficit and potassium humate on tuber yield and yield component of potato cultivars in Ardabill region. *Iran. Res. J. Environ. Sci.* **3,** 351–356.

Havlova, M., Dobrev, P. I., Motyka, V., Storchova, H., Libus, J., Dobra, J., Malbeck, J., Gaudinova, A., and Vankova, R. (2008). The role of cytokinins in responses to water-deficit in tobacco plants over-expressing trans-zeatin O-glucosyltransferase under 35S or SAG12 promoters. *Plant Cell Environ.* **31,** 341–353.

Heikal, M. M., Shaddad, M. A., and Ahmed, A. M. (1982). Effect of water stress and gibberellic acid on germination of flax, sesame and onion seeds. *Biol. Plant.* **24,** 24–29.

Henke, W., Herdel, K., Jung, K., Schnorr, D., and Loening, S. A. (1997). Betaine improves the PCR amplification of GC-rich DNA sequences. *Nucleic. Acids Res.* **25,** 3957–3958.

Hildebrand, D. F., and Grayburn, W. S. (1991). Lipid metabolites: Regulators of plant metabolism. *In* "Plant Biochemical Regulators" (H. W. Gausman, Ed.), pp. 69–98. Marcel Dekker Inc., NY, USA.

Hsu, S. Y., Hsu, Y. T., and Kao, C. H. (2003). The effect of polyethylene glycol on proline accumulation in rice leaves. *Biol. Plant.* **46,** 73–78.

Hu, Y., and Schmidhalter, U. (2005). Drought and salinity: A comparison of their effects on the mineral nutrition of plants. *J. Plant Nutr. Soil Sci.* **168,** 541–549.

Hu, C. A. A., Delauney, A. J., and Verma, D. P. S. (1992). A bifunctional enzyme (D1-pyrroline-5-arboxylate synthetase) catalyzes the first two steps in proline biosynthesis in plants. *Proc. Natl. Acad. Sci. USA* **89,** 9354–9358.

Huang, D., Jaradat, M. R., Wu, W., Ambrose, S. J., Ross, A. R., Abrams, S. R., and Cutler, A. J. (2007). Structural analogs of ABA reveal novel features of ABA perception and signaling in *Arabidopsis*. *Plant J.* **50,** 414–428.

Huang, D., Wu, W., Abrams, S. R., and Cutler, A. J. (2008). The relationship of drought-related gene expression in *Arabidopsis thaliana* to hormonal and environmental factors. *J. Exp. Bot.* **59,** 2991–2997.

Huerta, L., Garcia-Lor, A., and Garcia-Martinez, J. L. (2009). Characterization of gibberellin 20-oxidases in the citrus hybrid *Carrizo citrange*. *Tree Physiol.* **29,** 569–577.

Hussain, M., Malik, M. A., Farooq, M., Ashraf, M. Y., and Cheema, M. A. (2008). Improving drought tolerance by exogenous application of glycinebetaine and salicylic acid in sunflower. *J. Agron. Crop Sci.* **194,** 193–199.

Huttermann, A., Zommorodi, M., and Reise, K. (1999). Addition of hydrogels to soil for prolonging the survival of *Pinus helpensis* seedlings subjected to drought. *Soil Tillage Res.* **50,** 295–304.

Iqbal, M., and Ashraf, M. (2005). Changes in growth, photosynthetic capacity and ionic relations in spring wheat (*Triticum aestivum* L.) due to pre-sowing seed treatment with polyamines. *Plant Growth Regul.* **46,** 19–30.

Iqbal, M., and Ashraf, M. (2007). Seed treatment with auxins modulates growth and ion partitioning in salt-stressed wheat plants. *J. Integr. Plant Biol.* **49,** 1003–1015.

Iqbal, N., Ashraf, M. Y., and Ashraf, M. (2005). Influence of water stress and exogenous glycinebetaine on sunflower achene weight and oil percentage. *Int. J. Environ. Sci. Technol.* **2,** 55–60.

Iqbal, N., Ashraf, M., and Ashraf, M. Y. (2008). Glycinebetaine, an osmolyte of interest to improve water stress tolerance in sunflower (*Helianthus annuus L.*): water relations and yield. South Afr. *J. Bot.* **74,** 274–281.

Irget, M. E., Aksoy, U., Okura, B., Ongun, A. R., and Tepecik, M. (2008). Effect of calcium based fertilization on dried fig (*Ficus carica* L. cv. Sarılop) yield and quality. *Sci. Hort.* **118,** 308–313.

Jaleel, C. A., Gopi, R., Lakshmanan, G. M. A., and Panneerselvam, R. (2006). Triadimefon induced changes in the antioxidant metabolism and ajmalicine production in *Catharanthus roseus* (L.) G. Don. *Plant Sci.* **171,** 271–276.

Jaleel, C. A., Manivannan, P., Sankar, B., Kishorekumar, A., Gopi, R., Somasundaram, R., and Panneerselvam, R. (2007). Induction of drought stress tolerance by ketoconazole in *Catharanthus roseus* is mediated by enhanced antioxidant potentials and secondary metabolite accumulation. *Colloids Surf., B* **60,** 201–206.

Jaleel, A. C., Sankar, B., Murali, P. V., Gomathinayagam, M., Lakshmanan, G. M. A., and Panneerselvam, R. (2008). Water deficit stress effects on reactive oxygen metabolism in *Catharanthus roseus*; impacts on ajmalicine accumulation. *Colloids Surf., B* **62,** 105–111.

Jewer, P. C., Neales, T. F., and Incoll, L. D. (1985). Stomatal responses to carbon dioxide of isolated epidermis from a C_3 plant, the Argenteum mutant of *Pisum sativum* L., and a crassulacean-acid-metabolism plant *Kalanchoe daigremontiana* Hamet and Perr. *Planta* **164,** 495–500.

Jubany-Mari, T., Els, P., Sergi, M. B., and Leonor, A. (2010). The timing of methyl jasmonate, hydrogen peroxide and ascorbate accumulation during water deficit and subsequent recovery in the Mediterranean shrub *Cistas albidus* L. *Environ. Exp. Bot.* **69,** 47–55.

Kamountsis, A. P., and Chronopoulon-Sereli, A. G. (1999). Paclobutrazol affects growth and flower bud production in gardenia under different light regimes. *Hort. Sci.* **34,** 674–675.

Kamran, M., Shahbaz, M., Ashraf, M., and Akram, N. A. (2009). Alleviation of drought-induced adverse effects in spring wheat (*Triticum aestivum* L.) using proline as a pre-sowing seed treatment. *Pak. J. Bot.* **41,** 621–632.

Karakas, B., Ozias-Akins, P., Stushnoff, C., Suefferheld, M., and Rieger, M. (1997). Salinity and drought tolerance of mannitol-accumulating transgenic tobacco. *Plant Cell Environ.* **20,** 609–616.

Kasukabe, Y., He, L., Nada, K., Misawa, S., Ihara, I., and Tachibana, S. (2004). Overexpression of spermidine synthase enhances tolerance to multiple environmental stress and up-regulates the expression of various stress-regulated genes in transgenic *Arabidopsis thaliana. Plant Cell Physiol.* **45,** 712–722.

Kaul, S., Sharma, S. S., and Mehta, I. K. (2008). Free radical scavenging potential of L-proline: Evidence from *in vitro* assays. *Amino Acids* **34,** 315–320.

Kaur, S., Gupta, A. K., and Kaur, N. (2002). Effect of osmo and hydro priming of chickpea seeds on the performance of the crop in the field. *Int. Chickpea Pigeonpea Newsl.* **9,** 15–27.

Kavi Kishor, P. B., Sangam, S., Amrutha, R. N., Sri Laxm, P., Naidu, K. R., Rao, K. R. S. S., Rao, S., Reddy, K. J., Theriappan, P., and Sreenivasulu, N. (2005). Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: Its implications in plant growth and abiotic stress tolerance. *Curr. Sci.* **88,** 424–428.

Kaya, M. D., Okeu, G., Atak, M., Cykyly, Y., and Kolsarycy, O. (2006). Seed treatments to overcome salt and drought stress during germination in sunflower (*Helianthus annuus* L.). *Eur. J. Agron.* **24,** 291–295.

Khan, H. R., McDonald, G. K., and Rengel, Z. (2004). Zinc fertilization and water stress affects plant water relations, stomatal conductance and osmotic adjustment in chickpea (*Cicer arientinum* L.). *Plant Soil* **267,** 271–284.

Khanna-Chopra, G., Chaturverdi, S., Aggarwal, P. K., and Sinha, S. K. (2006). Effect of potassium on growth and nitrate reductase during water stress and recovery in maize. *Physiol. Plant.* **49,** 495–500.

Khedr, A. H. A., Abbas, M. A., Wahid, A. A. A., Quick, W. P., and Abogadallah, G. M. (2003). Proline induces the expression of salt-stress responsive proteins and may improve the adaptation of *Pancratium maritimum* L. to salt-stress. *J. Exp. Bot.* **54,** 2553–2562.

Khripach, V., Zhabinskiim, V., and Groot, A. (2000). Twenty years of brassinosteroids: Steroidal plant hormones warrant better crops for the XXI century. *Ann. Bot.* **86,** 441–447.

Koda, Y. (1992). The role of jasmonic acid and related compounds in the regulation of plant development. *Int. Rev. Cytol.* **135,** 155–199.

Kopyra, M., and Gwozdz, E. A. (2003). Antioxidant enzymes in paraquat and cadmium resistant cell lines of horseradish. *Biol. Lett.* **40,** 61–69.

Kuehn, G. D., and Phillips, G. C. (2005). Role of polyamines in apoptosis and other recent advances in plant polyamines. *Crit. Rev. Plant Sci.* **3,** 145–155.

Kufner, I., and Koch, W. (2008). Stress regulated members of the plant organic cation transporter family are localized to the vacuolar membrane. *BMC Res. Notes* **1,** 43.

Kumar, C. S., Wing, R. A., and Sundaresan, V. (2008). Efficient insertional mutagenesis in rice using the maize En/Spm elements. *Plant J.* **44,** 879–892.

Lechowski, Z. (1997). Stomatal response to exogenous cytokinin treatment of the hemiparasite *Melampyrum arvense* L. before and after attachment to the host. *Biol. Plant.* **39,** 13–21.

Leonard, R. T., Nell, T. A., and Hoyer, L. (2005). Response of potted rose varieties to short-term ethylene exposure. *Acta Hort.* **669,** 373–380.

Leshem, Y. Y. (1981). Oxy free radicals and plant senescence. What's new? *Plant Physiol.* **12,** 1–4.

Levi, A., Ovnat, L., Paterson, A. H., and Saranga, Y. (2009). Photosynthesis of cotton near-isogenic lines introgressed with QTLs for productivity and drought related traits. *Plant Sci.* **177,** 88–96.

Levitt, J. (1980). Responses of Plants to Environmental Stresses, Water, Radiation, Salt and Other Stresses, Vol. II. 2nd edn. Academic Press, NY, USA.

Li, M. D., and Zheng, S. X. (1996). Influence of potassium nutrition on growth and drought resistance of maize. *Soil Fertil. Beijing* **4,** 10–12.

Liu, J. H., Nada, K., Honda, C., Kitashiba, H., Wen, X. P., Pang, X. M., and Moriguchi, T. (2006). Polyamine biosynthesis of apple callus under salt stress: Importance of arginine decarboxylase pathway in stress response. *J. Exp. Bot.* **57,** 2589–2599.

Lopez-Carbonell, M., Alegre, L., and van Onckelen, H. (1994). Effects of water stress on cellular ultrastructure and on concentrations of endogenous abscisic acid and indole-3-acetic acid in *Fatsia japonica* leaves. *Plant Growth Regul.* **14,** 29–35.

Lu, S., Sub, W., Li, H., and Guo, Z. (2009). Abscisic acid improves drought tolerance of triploid bermudagrass and involves H_2O_2-and NO-induced antioxidant enzyme activities. *Plant Physiol. Biochem.* **47,** 132–138.

Ludlow, M. M., and Muchow, R. C. (1990). A critical evaluation of traits for improving crop yield in water limited environments. *Adv. Agron.* **43,** 107–153.

Ma, Q. Q., Zou, Q., Li, Y. H., Li, D. Q., and Wang, W. (2004). Amelioration of the water status and improvement of the anti-oxidant enzyme activities by exogenous glycinebetaine in water stressed wheat seedlings. *Acta Agron. Sin.* **30,** 321–328.

Ma, L., Sun, N., Liu, X., Jiao, Y., Zhao, H., and Deng, X. W. (2005). Organ specific expression of *Arabidopsis* genome during development. *Plant Physiol.* **138,** 80–91.

Ma, Q., Wang, W., Li, Y., Li, D., and Zou, Q. (2006). Alleviation of photoinhibition in drought-stresses wheat (*Triticum aestivum*) by foliar-applied glycinebetaine. *J. Plant Physiol.* **163,** 65–175.

Ma, X. L., Wang, Y. J., Xie, S. L., Wang, C., and Wang, W. (2007). Glycinebetaine application ameliorates negative effects of drought stress in tobacco. *Russ. J. Plant Physiol.* **54,** 472–479.

Maggio, A., Miyazaki, S., Veronese, P., Fujita, T., Ibeas, J. I., Damsz, B., Narasimhan, M. L., Hasegawa, P. M., Joly, R. J., and Bressan, R. A. (2002). Does proline accumulation play an active role in stress-induced growth reduction? *Plant J.* **31,** 699–712.

Mahmood, T., Ashraf, M., and Shahbaz, M. (2009). Does exogenous application of glycinebetaine as a pre-sowing seed treatment improve growth and regulate some key

physiological attributes in wheat plants grown under water deficit conditions? *Pak. J. Bot.* **41,** 1291–1302.
Makela, P., Peltonen-Sainio, P., Jokinen, K., Pehu, E., Setala, H., Hinkkanen, R., and Somersalo, S. (1998). Effect of foliar application of glycine betaine on stomatal conductance, abscisic acid and solute concentrations in leaves of salt- and drought-stressed tomato. *Aust. J. Plant Physiol.* **25,** 655–663.
Manivannan, P., Jaleel, C. A., Kishorekumar, A., Sankar, B., Somasundaram, R., Sridharan, R., and Panneerselvam, R. (2007). Changes in antioxidant metabolism of *Vigna unguiculata* (L.) Walp. by propiconazole under water deficit stress. *Colloids Surf., B* **57,** 69–74.
Manivannan, P., Jaleel, C. A., Somasundaram, R., and Panneerselvam, R. (2008). Osmoregulation and antioxidant metabolism in drought-stressed *Helianthus annuus* under triadimefon drenching. *C.R. Biol.* **331,** 418–425.
Mansfield, T. A., and Mc-Ainsh, M. R. (1995). Hormones as regulators of water balance. *In* "Plant Hormones" (P. J. Davies, Ed.), pp. 598–616. Kluwer Academic Publishers, Dordrecht, Berlin.
Marschner, H. (1995). Mineral Nutrition of Higher Plants. 2nd edn. Academic Press, London.
Marshall, J. G., Rutledge, R. G., Blumwald, E., and Dumbroff, E. B. (2000). Reduction in turgid water volume in jack pine, white spruce and black spruce in response to drought and paclobutrazol. *Tree Physiol.* **20,** 701–707.
Martin-Tanguy, J. (2001). Metabolism and function of polyamines in plants: Recent development (new approaches). *Plant Growth Regul.* **100,** 675–688.
Mathur, N., Singh, J., Bohra, S., Bohra, A., and Vyas, A. (2006). Improved productivity of mungbean by application of thiourea under arid conditions. *World J. Agric. Sci.* **2,** 185–197.
Mazher, A. A. M., Yaseen, A. A., and Zaghlou, S. M. (2007). Influence of foliar application of potassium on growth and chemical composition of *Bauhinia variegate* seedlings under different irrigation intervals. *World J. Agric. Sci.* **3,** 23–31.
McCue, R. F., and Hanson, A. D. (1990). Drought and salt tolerance: Towards understanding and application. *Trends Biotechnol.* **8,** 358–362.
McDonald, M. B. (2000). Seed priming. *In* "Seed Technology and Its Biological Basis" (M. Black and J. D. Bewley, Eds.), pp. 287–326. Sheffield Academic Press, England.
Mengel, K. (2002). Alternative or complementary role of foliar supply in mineral nutrition. *Acta Hort.* **594,** 33–47.
Michalczuk, L., Ribnicky, D. M., Cooke, T. J., and Cohen, J. D. (1992). Regulation of indole-3-acetic acid biosynthetic pathways in carrot cell cultures. *Plant Physiol.* **100,** 1346–1353.
Mirzapour, M. H., and Khoshgoftar, A. H. (2006). Zinc application effects on yield and seed oil content of sunflower grown on a saline calcareous soil. *J. Plant Nutr.* **29,** 1719–1727.
Mittler, R. (2002). Oxidatve stress, antioxidants and stress tolerance. *Trends Plant Sci.* **7,** 405–410.
Morgan, J. M. (1983). Osmoregulation as a selection criterion for drought tolerance in wheat. *Aust. J. Agric. Res.* **34,** 607–614.
Morgan, P. W. (1990). Effects of abiotic stresses on plant hormone systems. *In* "Stress Responses in Plants, Adaptation and Acclimation Mechanisms" (R. C. Alscher and J. R. Cumming, Eds.), pp. 113–146. Wiley-Liss Inc., NY.
Möttönen, M., Aphalo, P. J., and Lehto, T. (2001). Role of boron in drought resistance in Norway spruce (*Picea abies*) seedlings. *Tree Physiol.* **21,** 673–681.
Movahhedy-Dehnavy, M., Modarres-Sanavy, S. A. M., and Mokhtassi-Bidgolia, A. (2009). Foliar application of zinc and manganese improves seed yield and quality of safflower (*Carthamus tinctorius* L.) grown under water deficit stress. *Ind. Crops Prod.* **30,** 82–92.

Munne-Bosch, S., and Penuelas, J. (2003). Photo and antioxidative protection, and a role for salicylic acid during drought and recovery in field-grown *Phillyrea angustifolia* plants. *Planta* **217,** 758–766.

Munné-Bosch, S., Peñuelas, J., Asensio, D., and Llusià, J. (2004). Airborne ethylene may alter antioxidant protection and reduce tolerance of holm oak to heat and drought stress. *Plant Physiol.* **136,** 2937–2947.

Murata, N., Mohanty, P. S., Hayashi, H., and Papageorgiou, G. C. (1992). Glycinebetaine stablizes the association of extrinsic proteins with the photosynthetic oxygen-evolving complex. *FEBS Lett.* **296,** 187–189.

Murungu, F. S., Nyamugafata, P., Chiduza, C., Clark, L. J., and Whalley, W. R. (2003). Effects of seed priming, aggregate size, and soil matric potential on emergence of cotton (*Gossypium hirsutum* L.) and maize (*Zea mays* L.). *Soil Tillage Res.* **74,** 161–168.

Naidu, B. P., Cameron, D. F., and Konduri, S. V. (1998). Improving drought tolerance of cotton by glycinebetaine application and selection. "Proceedings of Australian Agronomy Conference, Australian society of Agronomy", pp. 355–358. The Regional Institute Publishing, Australia.

Nemeth, M., Janda, T., Horváth, E., Páldi, E., and Szalai, G. (2002). Exogenous salicylic acid increases polyamine content but may decrease drought tolerance in maize. *Plant Sci.* **162,** 569–574.

Nemhauser, J. L., and Chory, J. (2004). Bring it on: New insights into the mechanism of brassinosteroid action. *J. Exp. Bot.* **55,** 265–270.

Noiraud, N., Maurousset, L., and Lemoine, R. (2001). Transport of polyols in higher plants. *Plant Physiol. Biochem.* **39,** 717–728.

Norwood, M., Truesdale, M. R., Richter, A., and Scott, P. (2000). Photosynthetic carbohydrate metabolism in the resurrection plant *Craterostigma plantagineum*. *J. Exp. Bot.* **51,** 159–165.

Okuma, E., Soeda, K., Tada, M., and Murata, Y. (2000). Exogenous proline mitigates the inhibition of growth of *Nicotiana tabacum* cultured cells under saline conditions. *Soil Sci. Plant Nutr.* **46,** 257–263.

Ozdemir, F., Melike, B., Demiral, T., and Tűrkan, I. (2004). Effects of 24-epibrassinolide on seed germination, seedling growth, lipid peroxidation, proline content and antioxidative system of rice (*Oryza sativa* L.) under salinity stress. *Plant Growth Regul.* **42,** 203–211.

Pandey, D. M., Goswami, C., and Kumar, B. (2003). Physiological effects of plant hormones in cotton under drought. *Biol. Plant.* **47,** 535–540.

Papadakis, I. E., Protopapadakis, E., Therios, I. N., and Tsirakoglou, V. (2005). Foliar treatment of Mn deficient 'Washington navel' orange trees with two Mn sources. *Sci. Hort.* **106,** 70–75.

Papadakis, I. E., Sotiropoulos, T. E., and Therios, I. N. (2007). Mobility of iron and manganese within two citrus genotypes after foliar applications of iron sulfate and manganese sulfate. *J. Plant Nutr.* **30,** 1385–1396.

Patade, V. Y., Bhargava, S., and Suprasanna, P. (2009). Halopriming imparts tolerance to salt and PEG induced drought stress in sugarcane. *Agric. Ecosys. Environ.* **134,** 24–28.

Payne, W. A., Hossner, L. R., Onken, A. B., and Wedt, C. W. (1995). Nitrogen and phosphorus uptake in pearl millet and its relation to nutrient and transpiration efficiency. *Agron. J.* **87,** 425–431.

Percival, G. C., and Noviss, K. (2008). Triazole induced drought tolerance in horse chestnut (*Aesculus hippocastanum*). *Tree Physiol.* **28,** 1685–1692.

Pestana, M., Correia, P. J., de Varennes, A., Abadía, J., and Faria, E. A. (2001). The use of floral analysis to diagnose the nutritional status of oranges trees. *J. Plant Nutr.* **24,** 1913–1923.

Peterbauer, T., and Richter, A. (2001). Biochemistry and physiology of raffinose family oligosaccharides and galactosyl cyclitols in seeds. *Seed Sci. Res.* **11,** 185–197.

Picarella, M. E., Antonelli, M. G., Astolfi, S., Zuchi, S., Vernieri, P., and Soressi, G. P. (2007). Ethylene and ABA cross-communication and plant growth response to salt stress in tomato (*Solanum lycopersicum* L.). *In* "Advances in Plant Ethylene Research". (A. Ramina, Ed.), Proceedings of the 7th International Symposium on the Plant Hormone, Ethylene, pp. 113–114. Springer, Pisa, Italy.

Pill, W. G., and Necker, A. D. (2001). The effects of seed treatments on germination and establishment of kentucky bluegrass (*Poa pratensis* L.). *Seed Sci. Technol.* **29,** 65–72.

Prado, F. E., Boero, C., Gallardo, M., and Gonzalez, J. A. (2000). Effect of NaCl on germination, growth and soluble sugar content in *Chenopodium quinoa* Willd. seeds. *Bot. Bull. Acad. Sin.* **41,** 27–34.

Raun, W. R., and Johnson, G. V. (1999). Improving nitrogen use efficiency for cereal production. *Agron. J.* **92,** 357–363.

Ray, I. M., Segovia-Lerma, A., and Murray, L. W. (2004). Diallel analysis of carbon isotope discrimination and its association with forage yield among nine historically recognized alfalfa germplasm. *Crop Sci.* **44,** 1970–1975.

Rees, D., Sayre, K., Acevedo, E., Nava, S. T., Lu, Z., Zeiger, E., and Limon, A. (1993). Canopy temperatures of wheat, relationship with yield and potential as a technique for early generation selection.Wheat special report, No: 10 Cimmyt, Mexico.

Revenga, C., Brunner, J., Henninger, N., Kassem, K., and Payne, R. (2000). Pilot analysis of global ecosystems: Freshwater ecosystems. World Resource Institute, Washington, DC. 83 pp.

Rhodes, D., and Hanson, A. D. (1993). Quaternary ammonium and tertiary sulfonium compounds in higher plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **44,** 357–384.

Rhodes, D., Verslues, P. E., and Sharp, R. E. (1999). Role of amino acids in abiotic stress resistance. *In* "Plant Amino Acids: Biochemistry and Biotechnology" (B. K. Singh, Ed.), pp. 319–356. Marcel Dekker, NY.

Ribas-Carbo, M., Taylor, N. L., Giles, L., Busquets, S., and Patrick, M. (2005). Effects of water stress on respiration in soybean leaves. *Plant Physiol.* **139,** 466–473.

Riera, M., Valon, C., Fenzi, F., Giraudat, J., and Leung, J. (2005). The genetics of adaptive responses to drought stress: Abscisic acid-dependent and abscisic acid-independent signalling components. *Physiol. Plant.* **123,** 111–119.

Rimbaut, I. M., and Pilet, P. E. (1994). Water stress and indole-3-acetic acid of maize roots. *Planta* **193,** 502–507.

Ritchie, S., and Gilroy, S. (1998). Gibberellins: Regulating genes and germination. *New Phytol.* **140,** 363–383.

Rivero, R. M., Kojima, M., Gepstein, A., Sakakibara, H., Mittler, R., Gepstein, S., and Blumwald, E. (2007). Delayed leaf senescence induces extreme drought tolerance in a flowering plant. *Proc. Natl. Acad. Sci. USA* **104,** 19631–19636.

Roberts, M. F. (2005). Organic compatible solutes of halotolerant and halophilic microorganisms, successful favourable. *Saline Syst.* **1,** 1–30.

Rock, C. D., and Sun, X. (2005). Crosstalk between ABA and auxin signaling pathways in roots of *Arabidopsis thaliana* (L.) Heynh. *Planta* **222,** 98–106.

Rodriguez, R., and Redman, R. (2005). Balancing the generation and elimination of reactive oxygen species. *Proc. Natl. Acad. Sci. USA* **102,** 3175–3176.

Rodriguez, D., Goudriaan, J., Oyarzabal, M., and Pomar, M. C. (1996). Phosphorus nutrition and water stress tolerance in wheat plants. *J. Plant Nutr.* **19,** 19–39.

Rontein, D., Basset, G., and Hanson, A. (2002). Metabolic engineering of osmoprotectant accumulation in plants. *Metab. Eng.* **4,** 49–56.

Sajedi, N. A., Ardakani, M. R., Naderi, A., Madani, H., and Boojar, M. M. A. (2009). Response of maize to nutrients foliar application under water deficit stress conditions. *Am. J. Agric. Biol. Sci.* **4,** 242–248.

Salim, M. H., and Todd, G. W. (1968). Seed soaking as a pre-sowing, drought-hardening treatment in wheat and barley seedlings. *Agron. J.* **60,** 179–182.

Samarah, N., Mullen, R., and Cianzio, S. (2004). Size distribution and mineral nutrients of soybean seeds in response to drought stress. *J. Plant Nutr.* **27,** 815–835.

Sanchez-Bel, P., Egca, I., Romojaro, F., and Martínez-Madrid, M. (2008). Sensorial and chemical quality of electron beam irradiated almonds (*Prunus amygdalus*). *Food Sci. Technol.* **41,** 442–449.

Saneoka, H., Moghaieb, R. E. A., Premachandra, G. S., and Fujita, K. (2004). Nitrogen nutrition and water stress effects on cell membrane stability and leaf water relations in *Agrostis palustris* Huds. *Environ. Exp. Bot.* **52,** 131–138.

Sankar, B., Jaleel, C. A., Manivannan, P., Kishorekumar, A., Somasundaram, R., and Panneerselvam, R. (2007). Effect of paclobutrazol on water stress amelioration through antioxidants and free radical scavenging enzymes in *Arachis hypogaea* L. *Colloids Surf., B* **60,** 229–235.

Sankar, B., Jaleel, A. C., Manivannan, P., Kishorekumar, A., Somasundaram, R., and Panneerselvam, R. (2008). Relative efficacy of water use in five varieties of *Abelmoschus esculentus* (L.) Moench. under water-limited conditions. *Colloids Surf., B* **62,** 125–129.

Sarkar, D., Mandal, B., and Kundu, M. C. (2007). Increasing use efficiency of boron fertilisers by rescheduling the time and methods of application for crops in India. *Plant Soil* **301,** 77–85.

Saruhan, N., Turgut-Terzi, R., and Kadioglu, A. (2006). The effects of exogenous polyamines on some biochemical changes during drought stress in *Ctenanthe setosa* (Rosc.) Eichler. *Acta Biol. Hung.* **57,** 221–229.

Sarvas, M., Pavlenda, P., and Takáčová, E. (2007). Effect of hydrogel application on survival and growth of pine seedlings in reclamations. *J. Forest Sci.* **53,** 204–209.

Sembdner, G., and Parthier, B. (1993). The biochemistry and physiological and molecular actions of jasmonates. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **44,** 569–589.

Shad, K. K., John, G. M., and Leigh, W. M. (2001). Germination of soybean seed primed in aerated solution of polyethylene glycol 8000. *J. Biol. Sci.* **1,** 105–107.

Shahbaz, M., Ashraf, M., and Athar, H. R. (2008). Does exogenous application of 24-epibrassinolide ameliorate salt induced growth inhibition in wheat (*Triticum aestivum* L.)? *Plant Growth Regul.* **55,** 51–64.

Sharma, P. S., and Kumari, T. S. (1996). Effect of potassium under water stress on growth and yield of sorghum in Vertisol. *J. Potash Res.* **12,** 96–99.

Sharma, P. N., and Ramchandra, T. (1990). Water relations and photosynthesis in mustard plants subjected to boron deficiency. *Indian J. Plant Physiol.* **33,** 150–154.

Sharma, C. P., and Sharma, P. N. (1987). Mineral nutrient deficiencies affect plant water relations. *J. Plant Nutr.* **10,** 1637–1643.

Sharp, R. E. (2002). Interaction with ethylene: Changing views on the role of abscisic acid in root and shoot growth responses to water stress. *Plant Cell Environ.* **25,** 211–222.

Shen, B., Hohmann, S., Jensen, R. G., and Bohnert, H. J. (1999). Roles of sugar alcohols in osmotic stress adaptation: Replacement of glycerol by mannitol and sorbitol in yeast. *Plant Physiol.* **121,** 45–52.

Shinozaki, K., and Yamaguchi-Shinozaki, K. (1997). Gene expression and signal transduction in water-stress response. *Plant Physiol.* **115,** 327–334.

Shinozaki, K., and Yamaguchi-Shinozaki, K. (2000). Molecular responses to dehydration and low temperature: Differences and cross-talk between two stress signaling pathways. *Curr. Opin. Plant Biol.* **3,** 217–223.

Singh, D. K., and Sale, P. W. G. (2000). Growth and potential conductivity of white clover roots in dry soil with increasing phosphorus supply and defoliation frequency. *Agron. J.* **92,** 868–874.

Singh, S. P., Bargali, K., Joshi, A., and Chaudhry, S. (2005). Nitrogen resorption in leaves of tree and shrub seedlings in response to increasing soil fertility. *Curr. Sci.* **89,** 389–396.

Sitbon, F., and Perrot-Rechenmann, C. (1997). Expression of auxin-regulated genes. *Physiol. Plant.* **100,** 443–455.

Sitbon, F., Eklof, S., Riding, R. T., Sandberg, G., and Olsson, C. H. A. (1993). Little, patterns of protein synthesis in the cambial region of scots pine shoots during reactivation. *Physiol. Plant.* **87,** 601–608.

Smirnoff, N. (1993). The role of active oxygen in the water deficit and desiccation. *New Phytol.* **125,** 27–58.

Smirnoff, N., and Cumbes, Q. J. (1989). Hydroxyl radical scavenging activity of compatible solutes. *Photochemistry* **28,** 1057–1060.

Soeda, Y., Konings, M. C., Vorst, O., van Houwelingen, A. M., Stoopen, G. M., Maliepaard, C. A., Kodde, J., Bino, R. J., Groot, S. P. C., and van der Geest, A. H. M. (2005). Gene expression programs during *Brassica oleracea* seed maturation, osmopriming, and germination are indicators of progression of the germination process and the stress tolerance level. *Plant Physiol.* **137,** 354–368.

Sun, T. (2008). Gibberellin metabolism, perception and signaling pathways in Arabidopsis. *The Arabidopsis Book* **6,** 1–28.

Taiz, L., and Zeiger, E. (2002). Plant physiology. 3rd edn. Sinauer Associates, Sunderland.

Taiz, L., and Zeiger, E. (2006). Plant Physiology. 4th edn. Sinauer Associates, Sunderland.

Taji, T., Ohsumi, C., Iuchi, S., Seki, M., Kasuga, M., and Kobayashi, M. (2002). Important roles of drought- and cold-inducible genes for galactinol synthase in stress tolerance in *Arabidopsis thaliana. Plant J.* **29,** 417–426.

Taravati, A., Shokrzadeh, M., Ebadi, A. G., Valipour, P., Hassan, A. T. M., and Farrakhi, F. (2007). Various effects of sugar and polyols on the protein structure and function: Role as osmolyte on protein stability. *World Appl. Sci.* **2,** 353–362.

Thalooth, A. T., Tawfik, M. M., and Mohamed, H. M. (2006). A comparative study on the effect of foliar application of zinc, potassium and magnesium on growth, yield and some chemical constituents of mungbean plants grown under water stress conditions. *World J. Agric. Sci.* **2,** 37–46.

Tűrkan, M., Özdemir, B. F., and Koca, H. (2005). Differential responses of lipid peroxidation and antioxidants in the leaves of drought-tolerant *P. acutifolius* gray and drought-sensitive *P. vulgaris* L. subjected to polyethylene glycol mediated water stress. *Plant Sci.* **168,** 223–231.

Umar, S. (2006). Alleviating adverse effects of water stress on yield of sorghum, mustard and groundnut by potassium application. *Pak. J. Bot.* **38,** 1373–1780.

Umar, S., Bansal, S. K., and Umar, S. (1995). Potassium requirement of mustard (*Brassica juncea* L.) under moisture stress conditions. *Plant Physiol. Biochem.* **22,** 130–135.

Vanrensburg, L., Kruger, G. H. J., and Kruger, R. H. (1993). Proline accumulation, the drought tolerance selection: Its relantionship to membrane integrity and chloroplast ultra structure in *Nicotiana tabacum* L. *J. Plant Physiol.* **141,** 188–194.

Wang, S. Y. (1999). Methyl jasmonate reduces water stress in strawberry. *J. Plant Growth Regul.* **18,** 127–134.

Wang, W. B., Kim, Y. H., Lee, H. S., Kim, K. Y., Deng, X. P., and Kwak, S. S. (2009). Analysis of antioxidant enzyme activity during germination of alfalfa under salt and drought stresses. *Plant Physiol. Biochem.* **47,** 570–577.

Willis, L. G., and Piland, H. R. (1937). Some recent observations on the use of minor elements in North Carolina. *Soil Sci.* **44,** 251–263.

Woodward, A. W. (2005). Receptor for auxin. *Plant Cell* **17,** 2425–2429.

Xing, W., and Rajashekar, C. B. (1999). Alleviation of water stress in beans by exogenous glycinebetaine. *Plant Sci.* **148,** 185–192.

Yagmur, M., and Kaydan, D. (2008). Alleviation of osmotic stress of water and salt in germination and seedling growth of triticale with seed priming treatments. *Afr. J. Biotechnol.* **7,** 2156–2162.

Yamada, T., Takatsu, Y., Kasumi, M., Ichimura, K., and van Doorn, W. G. (2006). Nuclear fragmentation and DNA degradation during programmed cell death in petals of morning glory (*Ipomoea nil*). *Planta* **224,** 1279–1290.

Yamane, H., Sugawara, J., Suzuki, Y., Shimamura, E., and Takahashi, N. (1981). Synthesis of jasmonic acid and related compounds and their structure-activity relationships on the growth of rice seedlings. *Agric. Biol. Chem.* **44,** 2857–2864.

Yancey, P. H., Clark, M. E., Hand, S. C., Bowlus, R. D., and Somero, G. N. (1982). Living with water stress: Evolution of osmolyte systems. *Science* **217,** 1214–1222.

Yang, J., Zhang, J., Liu, K., Wang, Z., and Liu, L. (2007). Involvement of polyamines in the drought resistance of rice. *J. Exp. Bot.* **58,** 1545–1555.

Zeid, I. M., and Shedeed, Z. A. (2007). Alterations in nitrogen metabolites after putrescine treatment in alfalfa under drought stress. *Pak. J. Biol. Sci.* **10,** 1513–1518.

Zhang, J., Nguyen, H. T., and Blum, A. (1999). Genetic analysis of osmotic adjustment in crop plants. *J. Exp. Bot.* **50,** 291–302.

Zhang, M., Zhai, Z., Tian, X., Duan, L., and Li, Z. (2008). Brassinolide alleviated the adverse effect of water deficits on photosynthesis and the antioxidant of soybean (*Glycine max* L.). *Plant Growth Regul.* **56,** 257–264.

Zhan-Xiang, S., Jia-Ming, Z., and Wen-Tao, S. (2009). Coupled effects of soil water and nutrients on growth and yields of maize plants in a semi-arid region. *Pedosphere* **19,** 673–680.

Zhao, X. X., Ma, Q. Q., Liang, C., Fang, Y., Wang, Y. Q., and Wang, W. (2007). Effect of glycinebetaine on function of thylakoid membranes in wheat flag leaves under drought stress. *Biol. Plant.* **51,** 584–588.

Zhou, Y., Dong, Y. Z., Zan, S. P., and Sheng, F. (2006). Dynamic changes of physiological indexes in *Kalanchoe blossfediana* leaves under gradual drought stress of soil. *Xinjiang Agric. Sci.* **43,** 153–155.

Zou, M. H., Shi, C., and Cohen, R. A. (2002). High glucose via peroxynitrite causes tyrosine nitration and inactivation of prostacyclin synthase that is associated with thromboxane/prostaglandin H(2) receptor-mediated apoptosis and adhesion molecule expression in cultured human aortic endothelial cells. *Diabetes* **51,** 198–203.

CHAPTER SIX

Direct Seeding of Rice: Recent Developments and Future Research Needs

Virender Kumar *and* Jagdish K. Ladha

Contents

International Rice Research Institute, India office, Pusa, New Delhi, India

Advances in Agronomy, Volume 111
ISSN 0065-2113, DOI: 10.1016/B978-0-12-387689-8.00001-1

Abstract

Rice (*Oryza sativa* L.), a staple food for more than half of the world population, is commonly grown by transplanting seedlings into puddled soil (wet tillage) in Asia. This production system is labor-, water-, and energy-intensive and is becoming less profitable as these resources are becoming increasingly scarce. It also deteriorates the physical properties of soil, adversely affects the performance of succeeding upland crops, and contributes to methane emissions. These factors demand a major shift from puddled transplanting to direct seeding of rice (DSR) in irrigated rice ecosystems. Direct seeding (especially wet seeding) is

widely adopted in some and is spreading to other Asian countries. However, combining dry seeding (Dry-DSR) with zero/reduced tillage (e.g., conservation agriculture (CA)) is gaining momentum as a pathway to address rising water and labor scarcity, and to enhance system sustainability. Published studies show various benefits from direct seeding compared with puddled transplanting, which typically include (1) similar yields; (2) savings in irrigation water, labor, and production costs; (3) higher net economic returns; and (4) a reduction in methane emissions. Despite these benefits, the yields have been variable in some regions, especially with dry seeding combined with reduced/zero tillage due to (1) uneven and poor crop stand, (2) poor weed control, (3) higher spikelet sterility, (4) crop lodging, and (5) poor knowledge of water and nutrient management. In addition, rice varieties currently used for DSR are primarily selected and bred for puddled transplanted rice. Risks associated with a shift from puddled transplanting to DSR include (1) a shift toward hard-to-control weed flora, (2) development of herbicide resistance in weeds, (3) evolution of weedy rice, (4) increases in soil-borne pathogens such as nematodes, (5) higher emissions of nitrous oxide—a potent greenhouse gas , and (6) nutrient disorders, especially N and micronutrients. The objectives of this chapter are to review (1) drivers of the shift from puddled transplanting to DSR; (2) overall crop performance, including resource-use efficiencies of DSR; and (3) lessons from countries where DSR has already been widely adopted. Based on the existing evidence, we present an integrated package of technologies for Dry-DSR, including the identification of rice traits associated with the attainment of optimum grain yield with Dry-DSR.

1. Introduction

Rice is the world's most important crop and is a staple food for more than half of the world's population. Worldwide, rice is grown on 161 million hectares, with an annual production of about 678.7 million tons of paddy (FAO, 2009). About 90% of the world's rice is grown and produced (143 million ha of area with a production of 612 million tons of paddy) in Asia (FAO, 2009). Rice provides 30–75% of the total calories to more than 3 billion Asians (Khush, 2004; von Braun and Bos, 2004). To meet the global rice demand, it is estimated that about 114 million tons of additional milled rice need to be produced by 2035, which is equivalent to an overall increase of 26% in the next 25 years. The possibility of expanding the area under rice in the near future is limited. Therefore, this extra rice production needed has to come from a productivity gain. The major challenge is to achieve this gain with less water, labor, and chemicals, thereby ensuring long-term sustainability.

The Green Revolution technologies (the combination of higher-yielding cultivars, use of agrochemicals, including fertilizer, and irrigation) led to a rapid rise in rice yield, production, and area, which resulted in lower rice

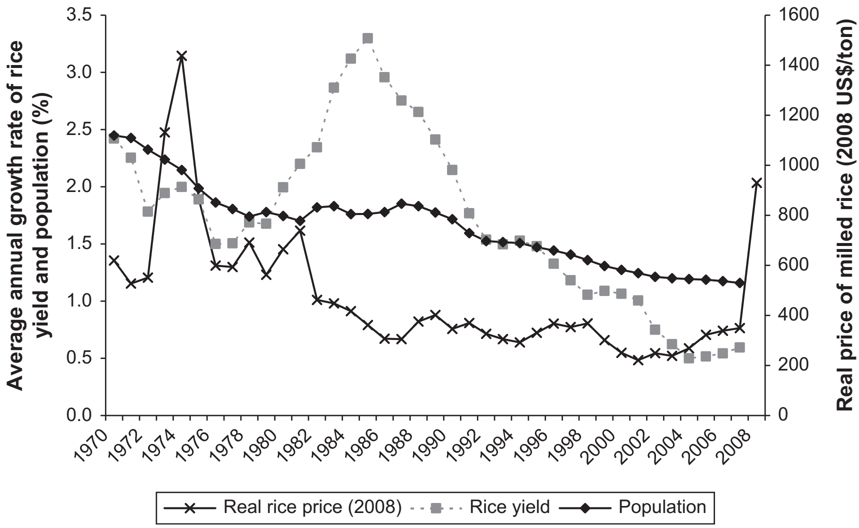


Figure 1 Trends of average annual growth rate of rice yield and population in Asia and world market rice price (1970–2008). (Rice price: 2008 is as of May 2008 price. Relate to Thai rice 5%-broken deflated by G-5 MUV Index deflator (adjusted based on April 17, 2008, data update). Source: www.worldbank.org.

prices, thereby benefiting poor consumers in rural and urban areas in Asia (Fig. 1). Although the overall increase in rice production has kept pace with population growth in Asia, growth in rice productivity has been declining since 1985 and, in more recent years, has fallen below the population growth rate (Fig. 1). If continued, this sluggish growth in rice productivity will cause significant imbalances between long-term supply and demand. In recent years, globally, consumption of rice surpassed production, which has led to the depletion of stocks. Current stocks are at their lowest since 1988 (IRRI, 2008). Because of all these factors, the long-term decline in rice price ended in 2001, with a sharp increase in 2008 to a level that had not been seen for decades (IRRI, 2008; Fig. 1).

The productivity and sustainability of rice-based systems are threatened because of (1) the inefficient use of inputs (fertilizer, water, labor); (2) increasing scarcity of resources, especially water and labor; (3) changing climate; (4) the emerging energy crisis and rising fuel prices; (5) the rising cost of cultivation; and (6) emerging socioeconomic changes such as urbanization, migration of labor, preference of nonagricultural work, concerns about farm-related pollution (Ladha *et al.*, 2009). Agronomic management and technological innovations are needed to address these issues in Asia.

In Asia, rice is commonly grown by transplanting seedlings into puddled soil (land preparation with wet tillage). Puddling benefits rice by reducing water percolation losses, controlling weeds, facilitating easy seedling

establishment, and creating anaerobic conditions to enhance nutrient availability (Sanchez, 1973). But, repeated puddling adversely affects soil physical properties by destroying soil aggregates, reducing permeability in subsurface layers, and forming hard-pans at shallow depths (Aggarwal *et al.*, 1995; Sharma and De Datta, 1985; Sharma *et al.*, 2003), all of which can negatively effect the following non-rice upland crop in rotation (Hobbs and Gupta, 2000; Tripathi *et al.*, 2005a). Moreover, puddling and transplanting require large amount of water and labor, both of which are becoming increasingly scarce and expensive, making rice production less profitable. Also, the drudgery involved in transplanting—a job largely done by women—is of serious concern. All these factors demand a major shift from puddled-transplanted rice production (CT-TPR) to direct seeding of rice (DSR) in irrigated areas. According to Pandey and Velasco (2005), low wages and adequate availability of water favor transplanting, whereas high wages and low water availability favor DSR. Depending on water and labor scarcity, farmers are changing either their rice establishment methods only (from transplanting to direct seeding in puddled soil [Wet-DSR]) or both tillage and rice establishment methods (puddled transplanting to dry direct seeding in unpuddled soil [Dry-DSR]).

Direct seeding can be categorized as (1) Wet-DSR, in which sprouted rice seeds are broadcast or sown in lines on wet/puddled soil, and (2) Dry-DSR, in which dry rice seeds are drilled or broadcast on unpuddled soil either after dry tillage or zero tillage or on a raised bed. Another category of DSR is water seeding, in which sprouted rice seeds are broadcast in standing water. Wet-DSR is primarily done to manage the labor shortage, and is currently practiced in Malaysia, Thailand, Vietnam, the Philippines, and Sri Lanka (Bhuiyan *et al.*, 1995; Pandey and Velasco, 2002; Weerakoon *et al.*, 2011). But, with the increasing shortages of water, the incentive to develop and adopt Dry-DSR has increased. Dry-DSR production is negligible in irrigated areas but is practiced traditionally in most Asian countries in rainfed upland ecosystems. Water seeding is widely practiced in the United States, primarily to manage weeds such as weedy rice, which are normally difficult to control (Hill *et al.*, 1991).

Both Dry- and Wet-DSR have the potential to reduce water and labor use compared with CT-TPR. Tabbal *et al.* (2002) in their on-farm studies in the Philippines observed on average 67–104 mm (11–18%) of savings in irrigation water in Wet-DSR compared with CT-TPR when irrigation application criteria was same for both establishment methods. Cabangon *et al.* (2002) in the Muda region of Malaysia found that irrigation water application in Dry-DSR was about 200 mm (40%) less than that in CT-TPR. Similarly, 10–50% savings in water have been claimed with Dry-DSR compared with CT-TPR from India when irrigation application criteria after crop establishment (CE) were either the appearance of hairline cracks or tensiometer-based (−20 kPa at 20-cm depth) (Bhushan *et al.*, 2007; Jat *et al.*, 2009; Sudhir-Yadav *et al.*, 2011a,b). Similar to saving in

water, DSR can reduce total labor requirements from 11% to 66% depending on season, location, and type of DSR compared with CT-TPR (Isvilanonda, 2002; Kumar *et al.*, 2009; Rashid *et al.*, 2009; Santhi *et al.*, 1998; Tisch and Paris 1994; Wong and Morooka, 1996). Labor requirements for CE decrease by more than 75% with direct seeding compared with transplanting (Dawe, 2005; Isvilanonda, 2002; Pandey and Velasco, 2002).

The way DSR is currently practiced differs considerably in different countries. Land preparation (tillage), establishment methods, seed rate, water management, weed management, and nutrient management vary from location to location. For example, seeding rates range from 20 to 60 kg ha^{-1} in South Asia to up to 200 kg ha^{-1} in some Southeast Asian countries (de Dios *et al.*, 2005; Gupta *et al.*, 2006; Guyer and Quadranti, 1985). Cleaning and plastering of bunds are an important component of field preparation for both weed and water management in Wet-DSR in Sri Lanka (Weerakoon *et al.*, 2011). A mix of traditional and modern practices based on farmers' long experiences and research innovations are being followed. Although a wealth of available information can lead us to develop DSR technologies that are suitable for wider agroecological conditions, more innovations are needed in the context of emerging challenges that future rice cultivation is likely to face.

During the past decade or so, there have been numerous efforts to find alternatives to the conventional practice of CT-TPR (Ladha *et al.*, 2009). Many of these studies have also considered ways to avoid or minimize extensive land preparation/tillage, which most farmers currently practice. In addition, there is a rich body of literature on case studies of DSR from countries where it is practiced widely. We believe that a systematic inventory and critical review of past and recent work would provide insight to enable us to develop efficient and viable rice production systems needed in the twenty first century. Therefore, the purpose of this review is to take stock of DSR. Specifically, we (1) analyze the reasons for a shift from puddled transplanting to different types of DSR, (2) summarize the current management practices of DSR in different countries, (3) compare the performance of different types of DSR with CT-TPR, (4) summarize the technological package of Dry-DSR including under zero tillage for major rice-based systems in South Asia, and (5) suggest future research needs for making direct-seeding systems more productive and sustainable. We aim to primarily target irrigated or favorable rainfed rice lowlands, which would continue to supply the growing rice demand (presently supplying 75% of world rice from about 50% of total rice area), and where the impact of shifts to DSR in saving of resources (i.e., labor and water) would be the greatest.

Various modifications of tillage/land preparation and CE are used to suit site-specific requirements. For the purpose of simplicity, these modifications are commonly referred to as alternative tillage/CE in this chapter. However, specific modifications are described when necessary.

2. Drivers of the Shift from Puddled Transplanting to Direct Seeding of Rice

2.1. Major drivers

2.1.1. Water scarcity

2.1.1.1. Current rice culture is a major freshwater user and is highly inefficient in its use Rice is a major user of freshwater because of its large area and consumption, which are, two to three times more than other cereals (Barker *et al*., 1998; Carriger and Vallée, 2007; Tuong *et al*., 2005). Rice consumes about 50% of total irrigation water used in Asia (Barker *et al*., 1998) and accounts for about 24–30% of the withdrawal of world total freshwater and 34–43% of the world's irrigation water (Bouman *et al*., 2007).

Conventional rice production systems (puddled transplanting) require large quantities of water. On average, 2500 l of water are applied, ranging from 800 to more than 5000 l, to produce 1 kg of rough rice (Bouman, 2009). The seasonal water input to rice fields is the combination of water used in land preparation and to compensate for evaporation, transpiration, seepage, and percolation losses during crop growth. Most of the water applied during crop growth is not used directly for transpiration, and is therefore considered lost from fields. Tuong and Bouman (2003) estimated seasonal water input for typical puddled transplanted rice to vary from 660 to 5280 mm depending on growing season, climatic conditions, soil type, and hydrological conditions, with 1000–2000 mm as a typical value in most cases. This consists of (1) 160–1580 mm for land preparation (puddling), with a typical value of 150–250 mm (Tuong, 1999); (2) 400–700 mm for evapotranspiration (ET) (600–700 mm in the dry season and 400–500 mm in the wet season); and (3) 100–3000 mm of unavoidable losses due to percolation and seepage (range of 100–500 mm for heavy clays and 1500–3000 mm for loamy/sandy soils). Tripathi (1990) studied seasonal water input to rice in India, which ranged from 1566 mm in a clay loam soil to 2262 mm in a sandy loam soil, with variations due primarily to deep percolation losses. Gupta *et al.* (2002) estimated water use for rice in the Indo-Gangetic Plains, which varied from 1144 mm in Bihar to 1560 mm in Haryana. In the Philippines, water use has been reported at 1300–1500 mm for the dry season and 1400–1900 mm for the wet season (Bouman *et al*., 2005).

The water productivity of rice in terms of ET is not different from other C_3 cereals such as wheat (Table 1). The higher water application in rice is mostly due to water requirements for puddling and losses associated with continuous flooding such as seepage and deep percolation losses to groundwater (Hafeez *et al*., 2007). Seepage and percolation losses vary from 25% to 85% of total water input depending on soil type and water table (25–50% in heavy soils with shallow water tables and 50–85% in coarse-textured soil

Table 1 Amount of water evapotranspired (liters) to produce one kilogram of major cereals

Crop	Photosynthesis type	Minimum	Maximum	Average	Median
			(L)		
Rice	C3	625	1667	917	980
Wheat	C3	588	1667	917	980
Maize	C4	370	909	556	625

Source: Zwart and Bastiaanssen (2004).

with deep water-table depth $\geq$1.5 m) (Cabangon *et al.*, 2004; Choudhury *et al.*, 2007; Dong *et al.*, 2004; Sharma *et al.*, 2002; Singh *et al.*, 2002a). Although the losses through seepage and percolation are often real for an individual farmer at the field level, they are often not as great at the basin scale since some water is recaptured and used downstream.

2.1.1.2. Water scarcity is increasing and availability of water for agriculture is decreasing Globally, water is becoming an increasingly scarce resource. In the major rice-growing Asian countries, per capita water availability decreased by 34–76% between 1950 and 2005 and is likely to decline by 18–88% by 2050 (Table 2). There are two key types of water scarcity: physical and economic. Physical scarcity occurs when the demand of the population exceeds the available water resources of a region. Economic water scarcity occurs when water is adequate, but is unavailable due to a lack of significant investment in water infrastructure (IWMI, 2000; Rijsberman, 2006).

Irrigated crop production increasingly faces competition for water from the other nonagriculture sectors. At present, irrigated agriculture accounts for 70% and 90% of total freshwater withdrawal globally and in Asia, respectively (Molden *et al.*, 2007; Tabbal *et al.*, 2002). The share of water for agriculture is declining fast, for which the reasons are often location specific, including (1) rising population, (2) falling groundwater table, (3) deteriorating water quality due to chemical pollution, salinization, etc., (4) inefficient irrigation systems, (5) changing food diet, and (6) competition with nonagricultural sectors (domestic, industrial, and environmental).

In Asia, the share of water in agriculture declined from 98% in 1900 to 80% in 2000 and is likely to further decline to 72% by 2020 (Fig. 2). In China, the water share in agriculture dropped from 88% in 1980 to 65% in 2005 and is likely to go down to 50% by 2050 (Fig. 2). Similarly, in other rice-growing Asian countries also, the share of water in agriculture is declining (Fig. 3). These data envisage significant transfers of water from irrigation to other sectors by 2050, thus warranting the development and deployment of highly water use efficient crop production technologies.

Table 2 Per capita water availability in major rice-growing countries of Asia (1950–2050)

Country	1950	1995	2000	2005	2010[a]	2015[a]	2020[a]	2025[a]	2050[a]
					m^3				
Bangladesh	56,411	19,936	16,744	15,393	14,335	13,452	12,703	12,086	10,593
China	5047	2295	2210	2134	2068	2006	1956	1927	1976
India	5831	2244	2000	1844	1717	1611	1525	1457	1292
Indonesia	31,809	12,813	12,325	11,541	10,881	10,361	9952	9609	8781
Japan	6541	4374	4317	4292	4307	4348	4423	4528	5381
Malaysia	74,632	22,642	19,593	17,790	16,336	15,179	14,242	13,503	11,497
Nepal	21,623	7923	6958	6245	5695	5230	4820	4470	3467
Pakistan	11,844	3435	3159	2822	2533	2277	2069	1900	1396
Philippines	15,390	4761	4158	3778	3450	3175	2945	2754	2210
South Korea	3247	1472	1424	1390	1363	1345	1336	1336	1500
Sri Lanka	5626	2410	2302	2212	2117	2041	1990	1961	1990
Thailand	8946	3073	2871	2714	2627	2559	2505	2465	2440
Vietnam	12,553	5095	4780	4472	4223	4015	3836	3684	3367

[a] Projections based on intermediate population growth rate.
Source: Modified from Gardner-Outlaw and Engelman (1997).

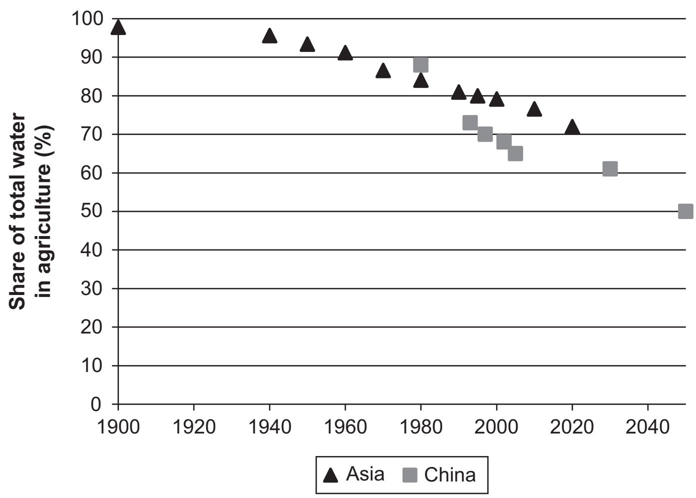


Figure 2 Agriculture's share of water use in Asia and China from 1900 to 2050. Source: State Hydrological Institute (2009) and Jiang (2009).

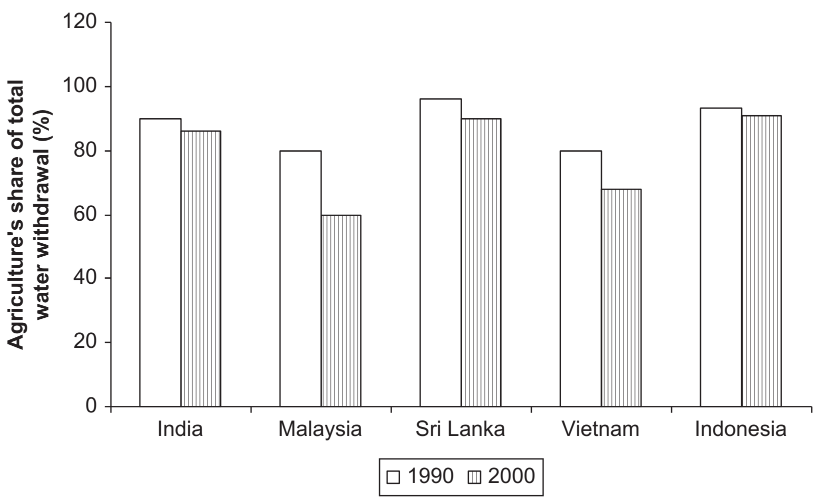


Figure 3 Agriculture's share of total water withdrawal in different rice-growing Asian countries in 1990 and 2000. Source: FAO AQUASTAT (2009).

Another evidence of growing water scarcity is the depleting groundwater resources, especially in South Asia and North China (Postel, 1997; Shah *et al.*, 2007), threatening the most intensive irrigated rice–wheat growing areas. Groundwater tables have fallen in the major rice-growing countries. In the Indian states of Punjab, Haryana, Gujarat, Tamil Nadu, Rajasthan,

Maharashtra, and Karnataka, it is falling at 0.5–2 m per year (Singh and Singh, 2002; Tuong and Bouman, 2003). In a recent study, jointly carried out by NASA and the German Aerospace Center (DLR), satellite data showed a groundwater table decline rate of 0.33 m per year in northwestern India (Rodell *et al.*, 2009; UC Irvine, 2009). The study estimated that over a period of 6 years (from August 2002 to October 2008), there was a net loss of 109 km^3 of groundwater in northern India, double the capacity of India's largest surface reservoir (Rodell *et al.*, 2009). In Bangladesh, because of heavy groundwater use, shallow wells are going dry by the end of the dry season (Ahmed *et al.*, 2004).

Similarly, in the North China Plains, many studies have reported increasing groundwater depletion (Bouman *et al.*, 2007; Liu and Yu, 2001; Xia and Chen, 2001). Water tables have dropped on average by 1–3 m per year in the region (Bouman *et al.*, 2007). In the western part of the 3-H basin, the groundwater table dropped from 3–4 m in the 1950s to 20 m in the 1980s and to 30 m in the 1990s (Liu and Xia, 2004). In China, groundwater overexploitation area has increased from 87,000 to 180,000 km^2 since the early 1980s (MWR, 2007).

The decline in the water table is mainly because of the heavy use of groundwater for irrigation as evidenced from intensive groundwater development (tubewells) during the past decades. Groundwater withdrawal structures and groundwater use in South Asian countries and China have increased rapidly (Table 3). For example, in India, the number of groundwater structures (dug wells and tubewells) increased from 3.9 million in 1950–1951 to more than 20 million in 2000 (Fig. 4) and they currently extract 185–210 km^3 $year^{-1}$ of groundwater (Table 3).

Increasing water scarcity has threatened the productivity and sustainability of the irrigated rice system in Asia (Tuong *et al.*, 2004). It is expected that the irrigated rice regions of South and Southeast Asia will experience some degree of water scarcity by 2025. About 13 million ha of Asia's irrigated wet-season rice and 2 million ha of irrigated dry-season rice may

Table 3 Number of groundwater structures (millions) and annual groundwater use (km^3 $year^{-1}$) in South Asian countries and China

Country	Groundwater structures (million)	Groundwater use (km^3 $year^{-1}$)
Bangladesh	0.80	31
China	3.50	75
India	20.00	185–210
Pakistan	0.80	45–55
Nepal Tarai	0.06	<1

Source: Deb Roy and Shah (2002), Shah (2005), and Qureshi *et al.* (2008).

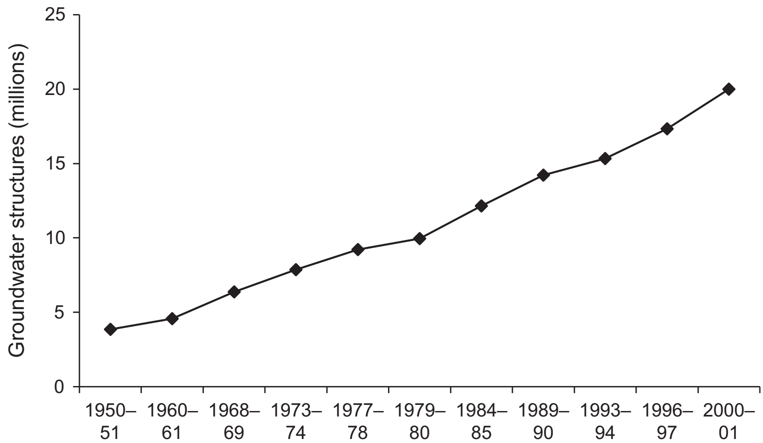


Figure 4 Groundwater withdrawal structures (millions) in India from 1950 to 2001. Sources: Adapted from Singh and Singh (2002) and Shah (2005).

experience physical water scarcity, and about 22 million ha of irrigated dry-season rice may suffer economic water scarcity by 2025 (Tuong and Bouman, 2003).

2.1.1.3. Water scarcity as a driver for direct seeding A grim water scenario in agriculture together with the highly inefficient rice production technologies currently adopted by a majority of farmers globally warrants the exploration of alternative rice production methods, which inherently require less water and are more efficient in water use. DSR provides some opportunities for saving water. Both Dry- and Wet-DSR are more water efficient and have an advantage over CT-TPR (Bhuiyan *et al*., 1995; Dawe, 2005; Humphreys *et al*., 2005; Tabbal *et al*., 2002). However, with increasing shortage of water, Dry-DSR with zero or minimal tillage in which potential savings of both labor and water can be much higher appears to have the greatest potential, especially for irrigated areas of Asia.

2.1.2. The labor shortage and increasing labor wages

CT-TPR is highly labor intensive. Both land preparation (puddling) and CE methods (transplanting) of CT-TPR require a large amount of labor. Rapid economic growth in Asia has increased the demand for labor in nonagricultural sectors, resulting in reduced labor availability for agriculture (Dawe, 2005; Fig. 5). For example, labor forces in agriculture are declining at 0.1–0.4%, with an average of 0.2% per year in Asia. In Bangladesh, Malaysia, and Thailand, the decline rate is much higher (0.25–0.40%), followed by India, the Philippines, and Cambodia (0.18%). In Bangladesh and Malaysia, the proportion of the labor force involved in agriculture dropped from 45% and

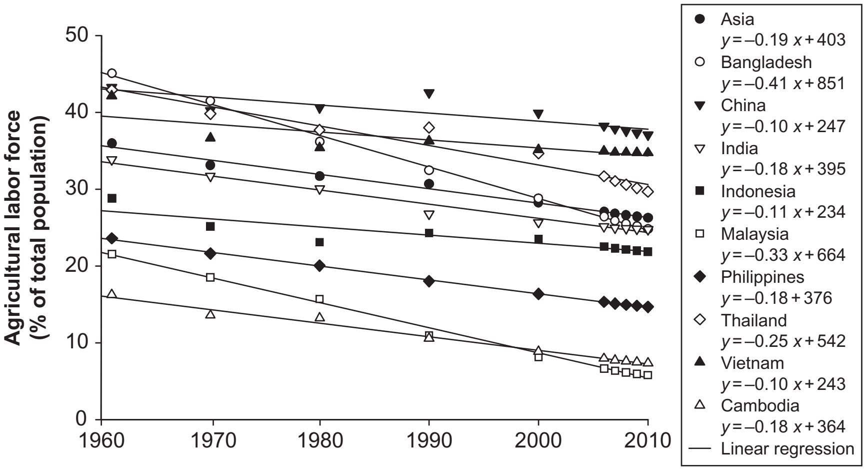


Figure 5 Agricultural labor force (% of total population) in selected Asian countries from 1960 to 2010. Source: IRRI World Rice Statistics database, available online http://beta.irri.org/solutions/index.php?option=com_content&task=view&id=250.

22% in 1961 to 25% and 6% in 2008, respectively. Similarly, in Thailand and Vietnam, the agricultural labor force dropped from 40% in the 1960s to 30–35% now. In addition, in the present changing socioeconomic environment in Asia, most people prefer nonagricultural work. Moreover, government policies such as The Mahatma Gandhi National Rural Employment Guarantee Act, introduced by the Indian government in 2005 (GOI, 2011), promising 100 days of paid work in people's home village, is creating a labor scarcity in the cereal bowl of northwest India, which is dependent on millions of migrant laborers from eastern Uttar Pradesh and Bihar for rice transplanting. Because of increasing labor scarcity, labor wages have gone up (e.g., shown in Fig. 6 for four Asian countries), which is making the CT-TPR production system uneconomical in many Asian countries. Because of high labor demand at the time of transplanting, increasing labor scarcity and rising wage rates are forcing farmers to opt for a shift in method of rice establishment from transplanting, which requires 25–50 person-days ha^{-1}, to direct seeding, which in comparison needs about 5 person-days ha^{-1} (Balasubramanian and Hill, 2002; Dawe, 2005).

2.2. Other drivers

2.2.1. Crop intensification and recent developments in DSR production techniques

Although labor and water are the major drivers for the shift from CT-TPR to DSR, economic incentives brought out by DSR through the integration of an additional crop (crop intensification) are another reason for the rapid

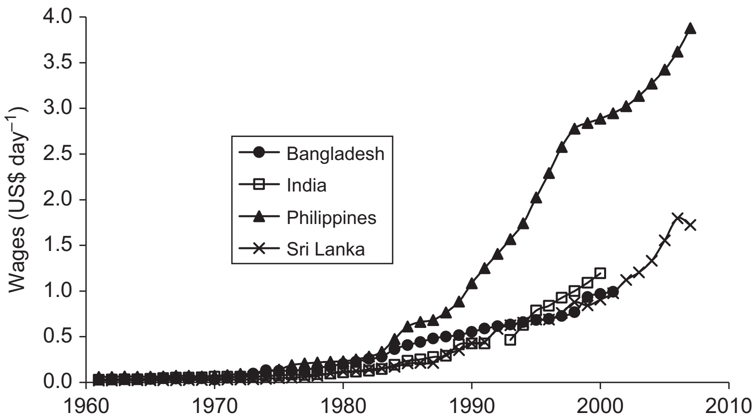


Figure 6 Trend of farm labor wages (US$ day^{-1}) in selected Asian countries from 1960 to 2007. Source: IRRI World Rice Statistics database, available online http://beta.irri.org/solutions/index.php?option=com_content&task=view&id=250.

spread/adoption of DSR in some regions. For example, in the Mekong Delta in Vietnam and Iloilo in the Philippines, DSR facilitated double cropping instead of a single crop of transplanted rice (Pandey and Velasco, 2002). Early establishment and short-duration varieties (95–105 days) permitted early harvesting of Dry-DSR in August, therefore, leaving enough time and rainfall to grow another rainfed crop of rice in Long An Province in the Mekong River Delta region of Vietnam. Some farmers can even grow a third crop of rice with supplemental irrigation during December to February (My *et al.*, 1995). DSR has gradually and steadily increased, covering almost 100% of the area, allowing double to triple crops in the region. Notably, the availability of high-yielding short-duration varieties and new herbicides for weed control largely made this shift technically viable (Mortimer *et al.*, 2008; Pandey and Velasco, 2002).

2.2.2. Adverse effects of puddling on soil physical properties and the succeeding non-rice crop

The adverse effects of puddling on soil quality, particularly on soil physical properties and on succeeding non-rice upland crops, are claimed to be other reasons for increased interest in shifting from CT-TPR to Dry-DSR on unpuddled soil or in zero-till conditions where an upland crop is grown after rice (Gopal *et al.*, 2010; Gupta *et al.*, 2006; Ladha *et al.*, 2009). This is especially relevant to the rice–wheat crop rotation, in which land goes through wetting and drying (Ladha *et al.*, 2003). Puddling results in a complete breakdown of soil aggregates, destruction of macropores, and formation of a hard pan at shallow depth. This practice benefits rice in

many ways, such as (1) easy weed control, (2) a reduction in deep percolation losses of water and nutrients, (3) ease of transplanting, (4) quick establishment of seedlings, and (5) improved nutrient availability (De Datta, 1981; Sanchez, 1973; Sharma and De Datta, 1985, 1986). Although puddling is known to be beneficial for growing rice, it can adversely affect the growth and yield of subsequent upland crops because of its adverse effects on soil physical properties, which includes poor soil structure, suboptimal permeability in the subsurface layer, and soil compaction (Aggarwal *et al.*, 1995; Gajri *et al.*, 1992, 1999; Gathala *et al.*, 2011; Kirchhof and So, 1996; Kumar *et al.*, 2008a; Meelu *et al.*, 1979). It is therefore important to identify alternatives to puddling (e.g., Dry-DSR), especially in those regions where water is becoming scarce and an upland crop is grown after rice.

The rice–wheat cropping system is practiced on about 18.5 million ha in South Asia and China (Dawe *et al.*, 2004; Ladha *et al.*, 2009), where wheat is grown in the cool and dry weather from November to March/April following rice during the warm and humid/subhumid season from June to October. In this region, many studies have reported on the adverse effect of puddling on the yield of a subsequent wheat crop (Arora *et al.*, 2006; Farooq *et al.*, 2008; Gangwar *et al.*, 2004; Gathala *et al.*, 2011; Hobbs *et al.*, 2002; Jat *et al.*, 2009; Tripathi *et al.*, 2005a,b).

The results of numerous published studies that evaluated the effects of puddling in rice on a subsequent wheat crop have been summarized in Table 4. On average, wheat yields were 9% higher when wheat was grown after Dry-DSR than when grown after CT-TPR. Of 35 studies, in 28 cases, puddling had adverse effects on succeeding wheat productivity (Table 4). Only one study (Singh *et al.*, 2001) reported a positive effect of puddling on the yield of a subsequent wheat crop, and five studies (Hobbs *et al.*, 2002; Malik *et al.*, 2005; McDonald *et al.*, 2006; Sharma *et al.*, 1995, 2005b) mentioned no effect.

Sharma *et al.* (2003) noted that the negative effect of puddling on wheat is more pronounced in medium- to fine-textured soils than in light-textured soils (sandy loam). However, other published results showed no clear relationship with soil type (Table 4). Aggarwal *et al.* (1995) and Kukal and Aggarwal (2003) reported intensity, depth, and duration of puddling as major determinants of effects on soil physical properties. Therefore, it is important to report site history, including the duration of puddling prior to experimentation for an accurate interpretation of the results. Unfortunately, most studies evaluating the effects of puddling on succeeding wheat have not reported site history.

In two medium-term studies conducted at Pantnagar on silty clay loam (5 years) and at Modipuram on sandy loam (7 years), the performance of wheat after either puddled or Dry-DSR was evaluated. The Pantnagar site had 12% higher wheat yield in Dry-DSR plots than in CT-TPR in all 5 years (Singh *et al.*, 2002b). However, at Modipuram, wheat yield was not

Table 4 Effects of tillage and rice establishment methods on grain yield of rice and subsequently grown wheat

S. no.	Location	Soil type	Tillage rice establishment method	Number of crop cycles	Rice yield[a]	Wheat yield[b] (kg ha^{-1})	Change (%) in wheat yield[c]	Reference
1	Pantnagar	Silty clay loam	CT-TPR[d]	2	–	3696 b[e]	0.0	Singh *et al.* (2004)
			Dry-DSR[f]		–	4029 a	9.0	
2	Pantnagar	Silty clay loam	CT-TPR	3	5655 a	3656 b	0.0	Tripathi *et al.* (2005a)
			Dry-DSR		5224 b	3944 a	7.9	
3	Pantnagar	Silty clay loam	CT-TPR	–	5650	4900	0.0	Tripathi (2002)
			Dry-DSR		4970	5500	12.2	
4	Pantnagar	Silty clay loam	CT-TPR	–	–	2890	0.0	Tripathi *et al.* (2005a)
			Dry-DSR		–	3300	14.2	
5	Pantnagar	Silty clay loam	CT-TPR	–	6356	3756	0.0	Tripathi *et al.* (2005a)
			Dry-DSR		6092	4350	15.8	
6	Pantnagar	Silty clay loam	CT-TPR	3	5486 a	3658 b	0.0	Sharma *et al.* (2005a)
			Dry-DSR		5024 b	3923 a	7.2	
7	Pantnagar	Silty clay loam	CT-TPR	2	5895 a	3560 b	0.0	Bajpai and Tripathi (2000)
			Dry-DSR		5380 a	4079 a	14.6	
8	Pantnagar	Silty loam	CT-TPR	3	6100 a	4100 b	0.0	Hobbs *et al.* (2002)
			Dry-DSR		5600 a	4600 a	12.2	
9	Pantnagar	Sandy loam	CT-TPR	6	5600 a	3900 a	0.0	Hobbs *et al.* (2002)
			Dry-DSR		5300 a	4000 a	2.6	
10	Pantnagar	Silty clay loam	CT-TPR	5	–	–	0.0	Singh *et al.* (2002b)
			Dry-DSR		–	–	12.0	
11	Pantnagar	Silty clay loam	CT-TPR	3	5224 a	3677 b	0.0	Sharma *et al.* (2004)
			Dry-DSR		5593 b	4018 a	9.3	

12	Pantnagar	Silty clay loam	CT-TPR	2	–	4170 b	0.0	Rath *et al.* (2000)
			Unpuddled-TPR		-	4640 a	11.3	
13	Pantnagar	Silty clay loam	CT-TPR	2	–	4665 b	0.0	Singh and Singh (2007)
			Dry-DSR		–	5055 a	8.4	
14	Modipuram	Sandy loam	CT-TPR	3	–	4653 b	0.0	Gangwar *et al.* (2004)
			Dry-DSR		–	5287 a	13.6	
15	Modipuram	Sandy loam	CT-TPR	4	7720 b	5000 b	0.0	Gangwar *et al.* (2009)
			Dry-DSR		8300 a	5710 a	14.2	
16	Modipuram	Loam	CT-TPR	2	3720 a	5370 a	0.0	Sharma *et al.* (1995)
			Dry-DSR		3620 a	5380 a	0.2	
17	Modipuram	Sandy loam	CT-TPR		4200 a	3860 b	0.0	Tomar *et al.* (2005)
			Dry-DSR		2310 b	4010 a	3.9	
18	Modipuram	Sandy loam	CT-TPR	2	7500 a	4350 b	0.0	Jat *et al.* (2009)
			Dry-DSR		6400 b	4787 a	10.0	
19	Modipuram	Silty loam	CT-TPR	7	8100 a	4760 b	0.0	Gathala *et al.* (2011)
			Dry-DSR		6820 b	5370 a	12.8	
20	Modipuram	Sandy loam	CT-TPR	2	4930 a	5060 a	0.0	Sharma *et al.* (2005b)
			Unpuddled-TPR		4900 a	5200 a	2.8	
21	Karnal	Clay loam	CT-TPR	2	–	4310 b	0.0	Tripathi *et al.* (2005b)
			Dry-DSR		–	4960 a	15.0	
22	Karnal	Clay loam	CT-TPR	2	–	–	0.0	Tripathi and Chauhan (2001)
			Dry-DSR		–	4500 a	9.0	

(*Continued*)

Table 4 (*Continued*)

S. no.	Location	Soil type	Tillage rice establishment method	Number of crop cycles	Rice yield[a]	Wheat yield[b] (kg ha^{-1})	Change (%) in wheat yield[c]	Reference
23	Karnal	Clay loam	CT-TPR		–	4330	0.0	Tripathi *et al.* (1999)
			Dry-DSR		–	4960	14.5	
24	Kaithal	Clay loam	CT-TPR	2	6530 a	4900 a	0.0	Malik *et al.* (2005)
			Dry-DSR		5430 b	5260 a	7.3	
25	Kaul	Clay loam	CT-TPR	2	7080 a	4630 b	0.0	Ram *et al.* (2006)
			Dry-DSR		4180 b	4900 a	5.8	
26	Kaul	Clay loam	CT-TPR	3	6780 a	4000 b	0.0	Dhiman *et al.* (1998)
			Dry-DSR		5910 b	4340 a	8.5	
27	Bilaspur	Clay loam	CT-TPR	3	5325 a	2980 b	0.0	Parihar (2004)
			Dry-DSR		4764 b	3236 a	8.6	
28	Ghaghraghat, Bahraich, UP	Sandy loam	CT-TPR	3	3333	2780	0.0	Singh *et al.* (2008)
			Dry-DSR		3045	3148	13.2	
29	New Delhi	Sandy clay loam	CT-TPR	3	4467 a	3700 a	0.0	Singh *et al.* (2001)
			Dry-DSR		3033 b	3333 b	−10.0	
30	Bhairahawa, Nepal	Silty clay loam	CT-TPR	2	5300 a	3100 b	0.0	Hobbs *et al.* (2002)
			Dry-DSR		5400 a	3400 a	9.7	
31	Khumaltar, Nepal	Silty loam	CT-TPR	2	6505	3733 a	0.0	McDonald *et al.* (2006)
			Dry-DSR		5710	3683 a	−1.3	

32	Pakistan	Fine silty	CT-TPR	2	4175 a	3910 b	0.0	Farooq *et al.* (2008)
			Dry-DSR		3340 b	4445 a	13.7	
33	Dinajpur, Bangladesh	Sandy loam	CT-TPR	4	3668 a	3768 a	0.0	Meisner *et al.* (2002)
			Dry-DSR		2353 b	3793 a	0.7	
		Average percent increase in yield of wheat grown after Dry-DSR					8.75 ± 0.99	

–, data not available.

[a] Rice yields are the averages of different years or tillage system.

[b] Wheat yields are the averages of different years or tillage system.

[c] Percent change in grain yield of wheat grown after Dry-DSR compared with grown after CT-TPR.

[d] CT-TPR, puddled transplanted rice.

[e] Different letters indicate a significant difference (pair comparison) at $P < 0.05$.

[f] Dry-DSR, dry direct-seeded rice in unpuddled soil.

Source: Modified from Kumar *et al.* (2008a).

different in the first 3 years followed by 0.5–1.0 t ha^{-1} (9–25%) higher yield in Dry-DSR plots in later years (Gathala *et al.*, 2011).

The main reason reported for the lower grain yield of wheat grown after CT-TPR was poor root development in a suboptimal soil physical environment resulting from puddling during the previous rice crop (Aggarwal *et al.*, 1995; Boparai *et al.*, 1992; Chenkual and Acharya, 1990; Ishaq *et al.*, 2001; Oussible *et al.*, 1992). Sadras and Calvino (2001) reported 0.4% lower wheat yield with every centimeter reduction in rooting depth. Ishaq *et al.* (2001) observed that subsoil compaction resulted in a reduction in both water and nutrient use efficiency in wheat by 38% owing to decreased root length. A greater reduction in root growth of wheat was observed in rice-based (e.g., rice–wheat) than in maize-based (e.g., maize–wheat) cropping systems in a sandy loam soil (Sur *et al.*, 1981).

Poor establishment and yields have also been found in other upland crops grown after rice, including soybean in eastern Java (Adisarwanto *et al.*, 1989), chickpea and Indian mustard in India (Gangwar *et al.*, 2008), and mungbean in the Philippines and other Asian countries (IRRI, 1984; Mahata *et al.*, 1990; So and Woodhead, 1987; Varade, 1990; Woodhead, 1990). The physical limitations imposed by puddling were implicated as the major causes of the inferior performance of these upland crops following rice.

However, it is important to note that rice yields in most cases (16 of 24) were higher (8–80%) under CT-TPR than under Dry-DSR. Other studies reported no difference in rice yield between CT-TPR and Dry-DSR (Bajpai and Tripathi, 2000; Hobbs *et al.*, 2002; Sharma *et al.*, 1995, 2005b). This highlights an interesting case of conflict between two crops when grown in rotation with rice. The process of puddling provides many benefits to rice but adversely affects the growth and yield of the subsequent upland crop (i.e., wheat) because of its adverse effects, especially on soil physical properties. This requires an alternative tillage and CE method that provides optimal yield of all the crops in a rotation with maximal efficiency of resource use such as labor and water.

2.2.3. Rising interest in CA

Declining/stagnating crop and factor productivity and a deteriorating resource base in cereal systems such as rice–wheat have led to the promotion of conservation tillage-based agriculture. Conservation tillage involves zero or minimal tillage followed by row seeding using a drill. Conservation tillage, when utilizes crop residue as mulch with improved crop and resource management practices, is termed CA or integrated crop and resource management (ICRM) (Ladha *et al.*, 2009).

Zero tillage, which has been promoted in wheat in the rice–wheat system, is now practiced on about 3 million ha in the Indo-Gangetic Plains of South Asia (Gupta and Seth, 2007; Harrington and Hobbs, 2009). Zero

or reduced tillage has had a significant positive impact on wheat productivity, profitability, resource-use efficiency, and farmers' livelihood, especially in those areas where the rice harvest is normally delayed (Erenstein and Laxmi, 2008; Ladha *et al*., 2009). Wider adoption of zero tillage in wheat occurred because of a combination of both increased yields (3–12%, primarily from timely planting) and a reduction in production cost (US$37–92 ha^{-1}, primarily from avoiding tillage) (Erenstein and Laxmi, 2008; Gupta and Seth, 2007; Hobbs and Gupta, 2003). However, unlike wheat, rice continues to be widely grown under conventional intensive tillage (puddling) and CE (transplanting), which is not only resource use inefficient and energy intensive but also delays the planting of wheat. To realize the full benefits of zero tillage, which otherwise are lost by doing puddling in rice, serious efforts are being made to develop zero-tillage rice followed by zero-tillage wheat—commonly referred to as "double zero tillage."

3. Types of Direct-Seeded Rice

Rice can be established by four principal methods: Dry-DSR, Wet-DSR, water seeding, and transplanting. These methods differ from others either in land preparation (tillage) or CE method or in both. Dry-, wet-, and water-seeding, in which seeds are sown directly in the main field instead of transplanting rice seedlings, are commonly referred to as direct seeding. Direct seeding is the oldest method of rice establishment. Prior to the 1950s, direct seeding was most common, but was gradually replaced by puddled transplanting (Grigg, 1974; Pandey and Velasco, 2005; Rao *et al*., 2007).

As it often happens, basic prototype technologies, when introduced to farmers' fields, undergo various modifications to suit local needs and also to optimize the benefits (Ladha *et al*., 2009). There is now a lot of confusion in the terminology used for various versions of direct-seeding practices. Therefore, a standard terminology is essential to communicate better among different groups of stakeholders. Different practices of direct seeding in various ecologies/environments have been classified and compared based on land preparation method, seedbed condition, oxygen level in the vicinity of germinating seed, and methods of sowing (Table 5).

3.1. Dry direct seeding

In Dry-DSR, rice is established using several different methods, including (1) broadcasting of dry seeds on unpuddled soil after either zero tillage (ZT-dry-BCR) or conventional tillage (CT-dry-BCR), (2) dibbled method in a well-prepared field (CT-dry-dibbledR), and (3) drilling of seeds in rows after conventional tillage (CT-dry-DSR), reduced tillage using a power-

Table 5 Major methods of rice direct seeding in various rice ecologies/environments

	Direct-seeding method	Abbreviations	Brief description	Tillage	Seedbed conditions	Seed environment	Depth of seeding	Seeding method/ pattern	Seeding implements	Rice ecology/ environment
A	Dry seeding (Dry-DSR)									
1	Conventionally tilled (dry) broadcast rice	CT-dry-BCR	Land is ploughed, harrowed but not puddled, leveled, and then dry seeds are broadcast manually before the onset of monsoon to use rainfall more effectively. In some cases, seeds are covered with soil by shallow tillage or planking.	Conventional dry tillage	Dry soil (unpuddled)	Aerobic	Surface or 0–3 cm	Broadcasting/ random	Manual	Mostly rainfed upland and flood-prone; some rainfed lowland
2	Conventionally tilled (dry) dibbled rice	CT-dry-dibbledR	Land preparation is same as in CT-dry-BCR but seeds are sown by dibbling methods, placing five to six seeds manually at desired spacing. This is useful in identifying weedy rice	Conventional dry tillage	Dry soil (unpuddled)	Aerobic	1–3 cm	Dibbling/rows	Manual	Mostly rainfed upland and flood-prone; some rainfed lowland
3	Conventionally tilled (dry) drill-seeded rice	CT-dry-DSR	Land preparation is same as in CT-dry-BCR. But, dry seeds are drilled in rows (20-cm apart) in a well-prepared soil (dry or moist) and leveled, followed by one light irrigation	Conventional dry tillage	Dry soil (unpuddled)	Aerobic	2–3 cm	Drilling/rows	Seed-cum-fertilizer drill	Irrigated and favorable rainfed lowland

4	Reduced-tillage (dry) drill-seeded rice with a power-tiller-operated seeder (PTOS)	RT(PTOS)-dry-DSR	In this, PTOS tills the soil at shallow depth (4–5 cm) and drills rice seed at the same time in rows at adjustable distance (20-cm row spacing) in a single operation	Reduced dry tillage (one-pass operation)	Dry soil (unpuddled)	Aerobic	2–3 cm	Drilling/rows	PTOS	Irrigated and favorable rainfed lowland
5	Zero-till dry broadcast rice	ZT-dry-BCR	Fields are flush-irrigated to moisten the soil and allow weeds to germinate. After about 2 weeks, glyphosate/paraquat is applied to kill weeds. Then, rice seeds (pregerminated) are broadcast in moist soil, followed by a light irrigation, if needed	Zero tillage	Dry soil (unpuddled)	Aerobic	Surface	Broadcasting/random	Manual	Irrigated and favorable rainfed lowland
6	Zero-till dry drill-seeded rice	ZT-dry-DSR	Fields are flush-irrigated to moisten the soil and allow weeds to germinate. After about 2 weeks, glyphosate/paraquat is applied to kill weeds. Then, a zero-till drill seeder is used to seed rice in rows (20 cm apart) in moist or dry soil, followed by a light irrigation, if needed.	Zero tillage	Dry soil (unpuddled)	Aerobic	2–3 cm	Drilling/rows	Zero-till-cum-fertilizer drill	Irrigated and favorable rainfed lowland

(*Continued*)

Table 5 (*Continued*)

	Direct-seeding method	Abbreviations	Brief description	Tillage	Seedbed conditions	Seed environment	Depth of seeding	Seeding method/ pattern	Seeding implements	Rice ecology/ environment
7	Raised-bed dry drill-seeded rice	Bed-dry-DSR	A bed former-cum-zero-till drill is used to form 37-cm-wide raised beds and 30-cm-wide furrows in a well-prepared and pulverized soil and rice seeds are sown in rows on both sides of the beds (moist/dry). Frequent light irrigations are applied for quick and uniform germination	Furrow irrigated raised bed	Dry soil (unpuddled)	Aerobic	2–3 cm	Drilling/rows	Bed planter-cum-seed drill	Irrigated and favorable rainfed lowland
B	Wet seeding (Wet-DSR)									
	Wet seeding on soil surface									
8	Conventionally tilled (wet) broadcast rice on surface of puddled soil	CT-wet-BCR (surface)	Land is ploughed, puddled, and leveled; pregerminated seeds are sown by broadcasting manually (24-h soaking and 24-h incubation) or by motorized blower (with 24-h soaking and 12-h incubation) 1–2 days after puddling on the surface of puddled (wet) soil after drainage	Both dry and wet tillage (puddling)	Wet soil (puddled)	Mostly aerobic	Surface	Broadcasting/ random	Manual or motorized blower	Irrigated and favorable rainfed lowland

9	Conventionally tilled (wet) drum-sown rice on surface of puddled soil	CT-wet-DrumR (surface)	Land preparation is same as in CT-wet-BCR but pregerminated seeds (with 24-h soaking and 12-h incubation) are sown in rows (18–20 cm apart) on the surface of wet soil by using a drum seeder	Both dry and wet tillage (puddling)	Wet soil (puddled)	Mostly aerobic	Surface	Line sowing	Drum seeder	Irrigated and favorable rainfed lowland
	Wet seeding on subsurface/anaerobic wet seeding									
10	Conventionally tilled (wet) subsurface broadcast rice	CT-wet-BCR (subsurface)	Land is ploughed, puddled, and leveled; pregerminated seeds (with 24-h soaking and 24-h incubation) are sown by broadcasting (manually or by using motorized blower) on wet soil immediately after puddling and suspended mud is allowed to settle down and form a protective cover over the seeds sown	Both dry and wet tillage (puddling)	Wet soil (puddled)	Mostly anaerobic	0.5–1 cm	Broadcasting/ random	Manual or motorized blower	Irrigated and favorable rainfed lowland

(*Continued*)

Table 5 (*Continued*)

	Direct-seeding method	Abbreviations	Brief description	Tillage	Seedbed conditions	Seed environment	Depth of seeding	Seeding method/ pattern	Seeding implements	Rice ecology/ environment
11	Conventionally tilled (wet) drill-seeded rice using anaerobic seeder	CT-wet-DSR (subsurface)	Land is ploughed, puddled, and leveled; pregerminated seeds (with 24-h soaking and 12-h incubation) are drilled in rows 1–2 days after puddling by using an anaerobic seeder fitted with furrow opener and closer	Both dry and wet tillage (puddling)	Wet soil (puddled)	Mostly anaerobic	0.5–1 cm	Drilling/rows	Anaerobic seeder	Irrigated and favorable rainfed lowland
C	Water seeding									
12	Water seeding after dry tillage	Dry-water seeding	Land is dry ploughed, disked, harrowed, leveled but not puddled, and the seedbed is rougher (large clods) than dry seeding. Alternatively, a smooth seedbed is firmed with a grooving implement, which results in a grooved seedbed (2.5-cm depth) on 17.5-25-cm spacing. Pregerminated	Dry tillage	In standing water	Mostly anaerobic	Standing water of 5–10 cm	Broadcasting/ random	Manual or aircraft or motorized blower or tractor-mounted broadcast seeder	Irrigated lowland

			seeds (24-h soaking and 24-h incubation) are then broadcast either manually or using a motorized blower or by a tractor-mounted broadcast seeder with the aircraft in standing water of 10- to 15-cm depth							
13	Water seeding after wet tillage	Wet-water seeding	Land is ploughed, puddled, and leveled as in CT-wet-DSR. Then, pregerminated seeds as explained in dry-water seeding are broadcast in standing water	Dry and wet tillage (puddling)	In standing water	Mostly anaerobic	Standing water of 5–10 cm	Broadcasting/ random	Manual or aircraft or motorized blower or tractor-mounted broadcast seeder	Irrigated lowland

Source: Modified from Ladha *et al.* (2009).

tiller-operated seeder (PTOS) [RT (PTOS)-dry-DSR], zero tillage (ZT-dry-DSR), or raised beds (Bed-dry-DSR) (Table 5). For CT-dry-DSR and ZT-dry-DSR, a seed-cum-fertilizer drill is used, which, after land preparation or in zero-till conditions, places the fertilizer and drills the seeds. The PTOS is a tiller with an attached seeder and a soil-firming roller. It tills the soil at shallow depth (4–5 cm), sows the seeds in rows at adjustable row spacing, and covers them with soil and lightly presses the soil for better seed-to-soil contact, all in a single pass (Khan *et al*., 2009). For Bed-dry-DSR, a bed-planting machine is used, which, after land preparation, forms a bed (37-cm wide raised bed and 30-cm wide furrows), places fertilizer, and drills the seed on both sides of the bed in a single operation (Bhushan *et al*., 2007; Singh *et al*., 2009c). The seedbed condition is dry (unpuddled), and the seed environment is mostly aerobic; thus, this method is known as Dry-DSR. This method is traditionally practiced in rainfed upland, lowland, and flood-prone areas of Asia (Rao *et al*., 2007). However, recently, this method has been gaining importance in irrigated areas where water is becoming scarce. Drill seeding is preferred over broadcasting in irrigated or favorable rainfed areas in both developed and developing countries as it allows line sowing and facilitates weed control between rows, saves seeds and time, and provides better CE. However, in some situations, broadcasting is preferred even in irrigated areas, for example, in Arkansas of the United States, where broadcasting by using an aircraft is common on clay soils or in wet years, when speed of planting is important. In Dry-DSR, land preparation is done before the onset of monsoon, and seeds are sown before the start of the wet season to take advantage of pre-monsoon rainfall for CE and early crop growth.

3.2. Wet direct seeding

In contrast to Dry-DSR, Wet-DSR involves sowing of pregerminated seeds with a radicle varying in size from 1 to 3 mm on or into puddled soil. When pregerminated seeds are sown on the surface of puddled soil, the seed environment is mostly aerobic and this is known as aerobic Wet-DSR. When pregerminated seeds are sown/drilled into puddled soil, the seed environment is mostly anaerobic and this is known as anaerobic Wet-DSR. In both aerobic and anaerobic Wet-DSR, seeds are either broadcast [CT-wet-BCR (surface)] or sown in-line using a drum seeder [CT-wet-DrumR (surface)] (Khan *et al*., 2009; Rashid *et al*., 2009) or an anaerobic seeder [CT-wet-DSR (subsurface)] with a furrow opener and closer (Balasubramanian and Hill, 2002). In CT-wet-DSR (subsurface), seed coating with calcium peroxide to improve oxygenation around germinating seeds can be used. When manual broadcasting is done, seeds are soaked in water for 24 h followed by incubation for 24 h. However, when motorized broadcasting is done, the pregermination period is shortened (24-h soaking

and 12-h incubation) to limit root growth for ease of handling (easy flow of sprouted seeds) and to minimize damage, as is the case when a drum seeder is used for row seeding (Balasubramanian and Hill, 2002). A drum seeder is a simple manually operated implement for sowing rice seed on puddled soil. It consists of six drums, each 25 cm long and 55 cm in diameter, connected one after the other on an iron rod having two wheels at the two ends (Khan *et al*., 2009). For the motorized blower, a 3.5-hp mist blower/duster is used, attached with either a 1-m-long blow pipe or a 20- to 30-m-long shower blow pipe (Jaafar *et al*., 1995).

3.3. Water seeding

Water seeding has gained popularity in areas where red rice or weedy rice is becoming a severe problem (Azmi and Johnson, 2009). Aerial water seeding is the most common seeding method used in California (United States), Australia, and European countries to suppress difficult-to-control weeds, including weedy rice. This method is also becoming popular in Malaysia. In this method, pregerminated seeds (24-h soaking and 24-h incubation) are broadcast in standing water on puddled (Wet-water seeding) or unpuddled soil (Dry water seeding). Normally, seeds, because of their relatively heavy weight, sink in standing water, allowing good anchorage. The rice varieties that are used possess good tolerance of a low level of dissolved oxygen, low light, and other stress environments (Balasubramanian and Hill, 2002). In addition to irrigated areas, water seeding is practiced in areas where early flooding occurs and water cannot be drained from the fields.

4. Current Cultivation Practices for Direct-Seeded Rice: Case Studies of the United States, Sri Lanka, and Malaysia

The key cultivation practices of DSR widely used by farmers in the United States, Sri Lanka, and Malaysia are reviewed here with an aim to learn lessons from their experiences. In all three countries, more than 90% of the area is under direct seeding (Table 6). Table 6 provides a comparison of the major characteristics of current practices of DSR followed in the three countries.

4.1. The United States

In the United States, rice is grown in three major areas: (1) the Grand Prairie and Mississippi River delta of Arkansas and Louisiana, (2) the Gulf Coast areas of Louisiana and Texas, and (3) California (Hill *et al*., 1991). The total

Table 6 Comparison of direct-seeding rice production systems in the United States, Sri Lanka, and Malaysia

	United States[a]	Sri Lanka[b]	Malaysia[c]
Area (million ha)[d]	1.20	1.03	0.67
DSR area (%)[e]	100	> 93	> 95
Types of DSR	Dry-DSR 67% and Dry water seeding 33%	Wet- and Dry-DSR. Wet-DSR is dominant. Dry-DSR is only < 5%	Wet- and Dry-DSR. Mostly, it is Wet-DSR. Wet Water seeding is emerging
Average yield (t ha^{-1})[d]	7.7	4.2[f]	3.6
Growing season	March/April to September/October in Arkansas, California, Louisiana, Mississippi, Missouri, and Texas, whereas in Florida, it is grown from mid-February to late October	Maha (main/wet season): Late September to February; Yala (minor/dry season): Early April to early September	Main season (wet season): October to March; off season (dry season): April to September
Mechanization level	High	Less	Moderate
Soil puddling (wet tillage)	Not puddled in both water seeding and Dry-DSR	Puddled in Wet-DSR	Puddled in Wet-DSR and water seeding
Method of land leveling	Laser-aided	Water buffalo or two- or four-wheel tractors using a traditional wooden leveler (wooden plank) or a hand-held leveling board, a wooden blade of about 30″ × 6″ connected to a wooden handle	Motor grader, four-wheel tractor with a rear bucket or bulldozer with or without laser-beam control system
Method of seeding	Drill seeding in Dry-DSR; broadcasting using airplane in water seeding	Broadcasting manually	Broadcasting using knapsack-mounted motorized blower

Seed rate (kg ha^{-1})	70–100 kg ha^{-1} in drill seeding; 100–170 kg ha^{-1} in water seeding	70–220 kg ha^{-1}	120 kg ha^{-1} in Wet-DSR; 150 kg ha^{-1} in water seeding
Method of seed preparation	Dry seeds for drill seeding and sprouted seeds (24- to 36-h soaking and 18- to 24-h incubation) for water seeding	Sprouted seeds (24- to 48-h soaking and 48- to 72-h incubation)	Sprouted seeds (24- to 48-h soaking and 12- to24-h incubation)
Varietal selection and breeding targets	Direct seeding	Direct seeding and transplanting	Direct seeding and transplanting
Irrigation	Fully irrigated	Partially irrigated. Major granary area well irrigated	Partially irrigated
Water management	Dry drill seeding: precise and controlled with the help of precise field leveling and levees (bunds) and good drainage facility. Field is kept moist for optimal crop establishment followed by flooding with 5–10 cm until 2 weeks before harvest. Water seeding: water management ranges from (a) continuous flood, (b) delayed flood in which water is drained for 3–4 weeks after seeding before establishing permanent flood, and (c) pinpoint	Precise and controlled with the help of field leveling and bunds and good drainage system. Puddled water is drained before sowing. During early stage, intermittent irrigation to keep soil moist for optimal and uniform crop stand. About seven DAS, flooding of 1- to 2-cm depth is established to suppress germinating weeds. Water level is then gradually raised with the growth of rice plants	Precise and controlled with the help of proper field leveling, bunds, and drainage system. Puddled water is drained either before sowing or within a day or 2 days after sowing. Water management is similar to that in Sri Lanka

(*Continued*)

Table 6 (*Continued*)

	United States[a]	Sri Lanka[b]	Malaysia[c]
	flood in which water is drained only briefly for 3–5 days after seeding and then similar to continuous flood system		
Fertilizer management	All P, K, Zn, and S applied basal. N is applied either in two (pre-flood and mid-season between panicle initiation and differentiation) or three splits (basal or at seedling, pre-flood, and mid-season). Chlorophyll meter or N analysis of flag leaf is used for mid-season N application	All P is applied as basal. K either basal or in two splits (basal and PI stage). N is applied in three to four splits. After basal, topdressing is based on the status of leaf color	All P, K, and 2/3 N at 15 DAS and remaining N at panicle initiation stage. Chlorophyll meter or LCC is also used for topdressing N application
Major weed control strategies	Integrated weed management with widespread careful use of herbicide and continuous submergence	Integrated weed management with widespread use of herbicides	Integrated weed management with widespread use of herbicides
Herbicide research and development	Highly developed	Moderately developed	Moderately developed
Most common herbicides used	*Preemergence*: Thiobencarb, clomazone, pendimethalin, quinclorac, and molinate	*Preemergence*: Thiobencarb, oxadiazon, butachlor, and molinate	*Preemergence*: Pretilachlor, oxadiazon, molinate, and thiobencarb

	Postemergence: Propanil, bispyribac, penoxsulam, fenoxaprop, cyhalofop, halosulfuron, bensulfuron, carfentrazone, bentazone, triclopyr, acifluorfen, 2,4-D, imazethapyr for Clearfield Rice, and tank mix of propanil with molinate, bensulfuron, halosulfuron, pendimethalin, thiobencarb, triclopyr, bentazone, and bentazone + bensulfuron, etc.	*Postemergence:* Bispyribac, fenoxaprop, sethoxydim, mefenaset, ethoxysulfuron, propanil, MCPA, 2,4-D, oxadiazon + propanil, thiobencarb + propanil, butachlor + propanil, quinclorac + propanil, or propanil + molinate + propanil	*Postemergence*: Bispyribac, fenoxaprop, propanil, pyrazosulfuron, bensulfuron, cinosulfuron, metsulfuron, chlorimuron, 2,4-D, and tank mixture of quinclorac + bensulfuron, molinate + 2,4-D, or bensulfuron, propanil followed by 2,4-D or molinate
Use of herbicide-resistant rice	Imidazoline-resistant rice (IMI-rice) cultivars are widely used	Not used	Locally adapted IMI-rice cultivars MR 220CL1 and MR 220CL2 developed and commercialized in 2010[g]
Emerging issues	(a) Herbicide resistance (b) Shift in weed flora (c) Weedy rice	(a) Shift in weed flora (b) Weedy rice (c) Herbicide resistance	(a) Herbicide resistance (b) Shift in weed flora (c) Weedy rice

[a] Compiled from Hill *et al.* (1997), Slaton (2001), Way and Cockrell (2008), and Saichuk (2009).
[b] Pathinayake *et al.* (1991) and Weerakoon *et al.* (2011).
[c] Hiraoka and Ho (1996), Fuji and Cho (1996), Wah (1998), Karim *et al.* (2004), Azmi and Johnson (2009).
[d] FAO (2010). Data given are of year 2008 for the United States and Malaysia.
[e] Sri Lanka (Weerakoon *et al.*, 2011); Malaysia (Azmi, personal communication), United States (Hill *et al.*, 1991).
[f] Weerakoon *et al.* (2011).
[g] Crop Biotech Update, June 16, 2010 (www.isaaa.org/kc/cropbiotechupdate/article/default.asp?ID=6371).

area is about 1.2 million ha, with an average yield of 7.7 t ha^{-1} (Table 6). All the rice in the United States is direct seeded and can be classified into water seeding (33%) and Dry-DSR (67%), which is mostly drill seeding. In the United States, soil is not puddled like in most tropical countries. Rice production in the United States is highly mechanized, which involves the use of laser technology for precision land leveling, large tractors and heavy-duty implements to prepare seedbeds, aircraft for seeding and pest control, and self-propelled combines with half or full tracks for harvesting in muddy soils. Therefore, unlike Asia, the direct-seeding production system in the United States is the least labor dependent. Accounts of production technologies of dry direct drill seeding and water seeding in the United States summarized below and in Table 6 are adapted from the rice production handbook of California (Hill *et al*., 1997), Arkansas (Slaton, 2001), Texas (Way and Cockrell, 2008), and Louisiana (Saichuk, 2009).

Fields are precisely leveled using a laser leveler with about 0.2% slope to ensure proper drainage and precise water control for achieving a good crop stand. For dry drill-seeded rice, a weed-free, firm, and well-pulverized seedbed is prepared, which ensures adequate seed-to- soil contact for a uniform crop stand. For the reduced-till system, either a spring or a fall/autumn stale seedbed is practiced in which emerged weeds are killed with nonselective herbicides (paraquat or glyphosate or glufosinate) prior to rice sowing. In zero-till systems, rice is planted directly in the crop residues of the preceding crop, and weeds emerged prior to sowing are killed with nonselective herbicides. For water seeding, a rough and cloddy seedbed is preferred to prevent seeds and seedlings from drifting, and also to facilitate seedling anchorage. In recent years, an implement known as a groover (large "V" roller) is being used to make a corrugated surface for anchoring water-seeded rice.

Seeds at rates varying from 70 to 100 kg ha^{-1} are drilled at a shallow depth ($<$2.5 cm) to achieve a final plant population of 100–160 plants m^{-2}. A 10% higher seed rate is used in zero-till, and about a 20% higher seed rate is used when seed is broadcast. A much lower seed rate is used for a hybrid variety than for a conventional variety. Although drill seeding is a predominant method in Dry-DSR, broadcasting is preferred on clay soil and in wet years. A higher seed rate (100–170 kg ha^{-1}) is used in water seeding to compensate for greater seed loss due to standing water. In this method, pregerminated seeds are used, in which seeds are soaked in water for 24–36 h and then drained for 18–24 h for sprouting. Soaking helps increase seed weight by 25%, which in turn facilitates sinking to the soil surface and reduces seed floating on the soil surface. Sprouting also speeds up the rate of emergence.

High-yielding varieties have been developed through breeding specifically targeted for direct seeding, including zero-till. Almost all farmers use certified seeds, which ensure seed purity and high germination, and they are

free from weed seeds, including red rice. Because of the use of certified seeds coupled with the practice of water seeding, the problem of red rice is kept under control in California. Herbicide-resistant varieties (Clearfield Rice) are preferred by farmers in areas infested with red/weedy rice.

All rice is fully irrigated with precise and controlled water management. Levees (bunds) are formed at every 5–8-cm drop in elevation for good water control. Land leveling plays a major role in precise water management. In dry seeding, the field is kept moist in the early season to ensure optimal CE, followed by a permanent flood of 5–10 cm throughout the growing season. At panicle development, it is critical to maintain flood to avoid a yield penalty. In water-seeded rice, water management is categorized as (a) a continuous flood system, (b) delayed flood system, and (c) pinpoint flood system (Table 6). Pinpoint water management is the most common one.

In the dry drill-seeded system, all P, K, Zn, and S are applied as basal. Prior to 1995, a three-way split application of N was common (basal at seeding or seedling stage, at pre-flood, and at reproductive stage between panicle initiation and differentiation). However, recently, two splits instead of three are preferred (pre-flood and mid-season) because of more precise water management and planting of short-duration cultivars (Snyder and Slaton, 2001). A chlorophyll meter or N analysis of the flag leaf is also used to determine the need of mid-season N application (Snyder and Slaton, 2001).

Weeds are controlled in an integrated manner by employing mechanical, cultural (certified seeds, crop rotation, good seedbed, land leveling, and precise water management), and chemical practices. However, the availability of a range of pre-, delayed pre-, and postemergence herbicides has played a major role in keeping weeds under control in direct seeding. Early seasonal weed control is critical for DSR success; therefore, preemergence herbicides with residual effects are used for achieving initial good control. Evolution of resistance in weeds against the most commonly used herbicides for their control and red rice infestation are the major issues in Dry-DSR in the United States. In recent years, more and more Clearfield Rice technology (rice resistant to imazethapyr, a broad-spectrum herbicide) is practiced on a large area to overcome the constraints imposed by red rice and other weeds that have developed resistance to commonly used herbicides.

4.2. Sri Lanka

Based on the total annual rainfall, Sri Lanka is broadly divided into three climatic zones: dry zone (DZ), intermediate zone (IZ), and wet zone (WZ) with annual rainfall <1500, 1500–2500, and >2500, respectively. There are two rice-growing seasons: (a) *Maha* (main season) from late September to February, during which inter-monsoon and northeast monsoon rains are

well distributed all over the island, and (b) *Yala* (minor-season) from early April to early September, during which rain is mostly restricted to the southwest region. The area under rice cultivation is 0.7 million ha of which 0.6 and 0.4 million ha are being cultivated during the major and minor seasons, respectively, with an average yield of 4.2 t/ha (Table 6). Direct seeding is the predominant method (93%), with the majority under wet seeding, in which sprouted seeds are broadcast on puddled soil (CT-wet-BCR). The area under Dry-DSR is less than 5%, in which dry seeds are broadcast on dry unpuddled soil (CT-dry-BCR) (Table 6). The key production technologies of Wet-DSR in Sri Lanka are summarized in Table 6, which has largely been adapted from Pathinayake *et al.* (1991) and Weerakoon *et al.* (2011).

Cleaning and plastering of bunds followed by two ploughings and land leveling are the key land preparation and water management practices in Sri Lanka. These practices are traditionally developed and perfected by the farmers, and continue to be widely practiced. The cleaning and plastering of bunds help in (1) reducing weed incidence on bunds and their spread to the main field, (2) minimizing seepage of water and nutrients, and (3) ensuring good water management. With the onset of monsoon, fields are ploughed and puddled using either a two- or a four-wheel tractor, with buffaloes or manual land preparation in low-country WZs where fields are boggy or too wet, and the soil is too sticky. Fields are then leveled using either a water buffalo or a two- or four-wheel tractor in such a way that there is a small gradient toward the drainage outlet of the field. Leveling ensures good water control, including drainage, critical at the early stage for good and uniform CE. Tractor-mounted precision levelers are not used in Sri Lanka (Table 6). Excess standing water is drained, and seeds are broadcast manually on the same day of leveling if the soil is not very loose. Otherwise, sowing is delayed until the soil surface becomes a little harder.

Drainage is crucial for growing a successful Wet-DSR crop. This is achieved by preparing a network of primary, secondary, and tertiary drainage canals in the fields. After construction of the main (primary) drain, shallow and lateral (secondary and tertiary) drains are connected to the outlets to drain out the remaining water from the field. In addition, to ensure a good crop stand, seeds are processed to maximize germination and minimize weed seed contamination. For this, seeds of desired cultivars are cleaned to remove empty grains and chaff, followed by soaking in water to remove half-filled grains and for sprouting. Seed depth (1–2 cm) is also critical for good CE. The puddled soil is kept firm enough to keep broadcast seed partially buried in the soil. Farmers believe that, if the mud is too soft or dry, germination is adversely affected. A seed rate of 100 kg ha^{-1} is recommended but seed used by farmers varies from 70 to 220 kg ha^{-1}. Farmers using a high seed rate believe that this helps in suppressing weeds.

High-yielding medium-duration (105 days) varieties suitable for Wet-DSR are most commonly used by farmers except in the minor irrigation scheme and in the WZ where water is not a constraint and relatively longer duration cultivars of over 105 days are preferred. Another reason for using long-duration varieties in the WZ and in some areas of the IZ is to avoid heavy mid-season rain coinciding with flowering. Like in the United States, the breeding program targets direct seeding. All new rice lines are field-tested under a direct-seeded environment in different agroecological zones before recommending them to farmers. All the released varieties in Sri Lanka have adequate mechanisms of resistance against major diseases and insect pests, and lodging.

Water is precisely managed. During the early stage (after rice seed germination), fields are irrigated intermittently to avoid desiccation of rice seedlings and to ensure a uniform crop stand. Later, 7 days after sowing, fields are flooded to a depth of 1–2 cm to suppress germinating weeds. Water depth is gradually increased with the growth of rice plants.

All P is applied basal and K in two splits (basal and at panicle initiation). Nitrogen is applied in three or four splits. After basal application, topdressing is done based on the status of leaf color. But the quantity of N applied varies with the experience of rice farming and financial status of farmers.

Weeds are controlled by the integration of cultural and chemical methods. Weed pressure is minimized initially by land preparation and water management (shallow flooding of fields with water at seven DAS to suppress weed germination). Almost all the farmers depend on herbicides for weed control. The availability of effective and selective herbicides suitable for direct-seeding conditions in the country has played an important role in achieving good weed control. Two major issues that have emerged with the continuous use of Wet-DSR are (1) the evolution of herbicide resistance in weeds and (2) infestation of rice with weedy rice.

4.3. Malaysia

In Malaysia, rice is grown mainly in two seasons in a year: (i) the main/wet season from October to March and (ii) the off season from April to September (Table 6). The total cultivated area is 0.67 million ha, of which >95% is Wet-DSR. During the main season, rainfall is usually sufficient to meet the water requirement. But, in the off-season, the crop is irrigated from a nearby canal. Unlike Sri Lanka and somewhat similar to the United States, direct seeding is mechanized in Malaysia. Puddling, which was done earlier with draft animals, is replaced with pedestrian power tillers and four-wheel-drive tractors. Similarly, manual harvesting was common in the past but is now done by combines. The key production technologies of Malaysian Wet-DSR described here and presented in

Table 6 are adapted from Hiraoka and Ho (1996), Fuji and Cho (1996), Wah (1998), Karim *et al.* (2004), and Azmi and Johnson (2009).

Seeding is done on drained or shallow flooded puddled and precisely leveled fields by broadcasting pregerminated seeds using a knapsack-mounted motorized blower. When sowing is done in shallow standing water, the field is drained within a day or two to ensure a good crop stand as young plants can tolerate continuous flooding only up to a maximum of 2 or 3 days. Therefore, the provision to drain excess water is important in Wet-DSR to ensure a uniform and good establishment. Traditionally, drainage is achieved by making temporary or semi-permanent ditches of various sizes. Land leveling, considered a prerequisite, is carried out by using a tractor with a rear bucket or a motor grader with a laser control system. Water and fertilizer management are similar to those practiced in Sri Lanka.

In the beginning, when Wet-DSR had just started in Malaysia, yields used to be inconsistent and fluctuated. This was because of inadequate knowledge of land, crop, and water management, and the unavailability of suitable cultivars for DSR conditions. Subsequently, research and infrastructure improvement, especially of irrigation and cultural management, have led to higher yields. Besides, priority was given to developing cultivars specifically bred for direct-seeding conditions, which resulted in additional yield gains. Direct seeding, after its introduction in the late 1970s, has now emerged as a viable alternative to transplanting and has sustained rice production in the country. Crop lodging is still a problem in Wet-DSR, for which cultivar selection has been advocated. Like in Sri Lanka and the United States, in Malaysia too, there are reports of a shift in weed flora, the appearance of weedy rice, and resistance in weeds against herbicides.

5. The Performance of Direct-Seeded Rice Compared with Transplanted Rice

In this section, the performance of different types of DSR methods varying in tillage and method of establishment is compared with that of conventional puddled transplanted rice (CT-TPR). The performance criteria used included grain yield, irrigation water use, labor use, economics, and greenhouse gas (GHG) emissions. The data used for this analysis largely came from 215 studies (165 researcher-managed and 50 farmer-managed), from six major rice-growing Asian countries (India, Nepal, Pakistan, Bangladesh, the Philippines, and Thailand). Only those studies were considered in which a control (puddling followed by rice transplanting) was included for comparison. In studies in which other factors (e.g., planting dates, fertilizer level, weed control, water management, and genotypes) were evaluated, averages across all factors were considered. Likewise,

if the studies were conducted over several years, averages of all the years were used. As discussed earlier, farmers often modify technologies to suit their needs, and several variants of DSR practices were found in the literature. We grouped those in which minor modifications were made. Studies that did not provide a clear description of establishment methods (e.g., broadcast or drill seeding) were grouped into direct seeding. To compare performance, changes and percent change in various parameters were estimated for each study compared with CT-TPR.

Because of the unbalanced nature of the data, the analysis was performed using a mixed model procedure (SAS, 2001) with studies as replicates and a varying number of treatments in each replicate. Treatment effects were always considered as a fixed effect. For country-wise analysis, a study/replication was considered as a random effect, whereas, for combined analysis, country, replication/study nested within a country [replication (country)], and treatment × country were taken as random effects. For yield, treatment × country interaction was significant but, for cost and net income, the interaction was nonsignificant; therefore, only treatment (fixed effect) and replication (random effect) were included in the model statement for cost and net income analysis. The analysis estimated an adjusted mean of the original, change, and percent change data for each treatment. However, all interpretations were made on an adjusted mean of change data only and treatment means were compared against the control (puddled transplanted rice) at the 5% level of significance. A negative change indicated a lower value in DSR treatment than in CT-TPR, and the reverse was true when there was a positive change.

5.1. Rice grain yield

Our analysis showed that the performance of different types of DSR methods varied with countries as suggested by significant country × treatment (tillage/CE) interaction ($P = 0.023$). In India, yields were significantly lower (9.2–28.5%) in Dry-DSR than in CT-TPR (Table 7 and Fig. 7). Yields of Bed-dry-DSR were lower by 29%, whereas those of CT-dry-DSR and ZT-dry-DSR were lower by 9.2–10.3%. In Pakistan, yields of both Wet- and Dry-DSR were 12.7–21.0% lower than CT-TPR. In Bangladesh and the Philippines, yields of CT-wet-DSR were higher (8.6–18.5%) than those of CT-TPR, whereas in all other countries, yields were similar to those of CT-TPR. In general, line/drill seeding (compared with broadcasting) and Wet-DSR (compared with Dry-DSR) yielded more.

Apart from the six countries we analyzed, Wet-DSR performed similar to CT-TPR in Cambodia (Rickman *et al.*, 2001). Similarly, Mitchell *et al.* (2004) reported that DSR performed similar to CT-TPR also in Laos, Thailand, and Cambodia.

Table 7 Analysis of rice grain yield comparisons between conventional puddled transplanting and various alternative tillage and crop establishment methods in Asia

Country	Tillage and CE methods[a]	*N*[b]	Adjusted mean yield (t ha^{-1})[c]	Δ Yield (t ha^{-1})[d]	*P* value[e]	% Change
India	CT-TPR	98	5.48	–	–	–
	CT-wet-BCR	26	5.12	−0.39	0.0056	−7.5
	CT-wet-DSR	35	5.34	−0.10	NS	−1.9
	CT-dry-BCR	3	4.18	−1.20	0.0005	−26.5
	CT-dry-DSR	66	4.95	−0.53	<0.0001	−9.2
	Bed-dry-DSR	22	3.65	−1.75	<0.0001	−28.5
	ZT-dry-DSR	19	4.86	−0.65	<0.0001	−10.3
Bangladesh	CT-TPR	30	5.30	–	–	–
	CT-wet-BCR	16	5.45	0.12	NS	2.9
	CT-wet-DSR	12	5.66	0.46	0.0010	8.6
	ZT-dry-BCR	4	5.24	−0.24	NS	−2.0
	ZT-dry-DSR	6	5.50	0.08	NS	2.3
Pakistan	CT-TPR	12	3.95	–	–	–
	CT-wet-seeding (CT-wet-BCR, CT-wet-DSR)	3	3.06	−0.86	0.0016	−19.8
	CT-dry-DSR	10	3.40	−0.55	0.0045	−12.7
	Bed-dry-DSR	5	3.42	−0.53	0.0156	−12.7
	ZT-dry-DSR	3	3.12	−0.90	0.0011	−21.0
Nepal	CT-TPR	14	4.80	–	–	–
	CT-wet-seeding (CT-wet-BCR, CT-wet-DSR)	6	5.00	0.24	NS	5.5
	CT/RT-dry-DSR	15	4.80	0.00	NS	0
	Bed-dry-DSR	3	4.55	−0.29	NS	−4.6
Philippines	CT-TPR	33	5.94	–	–	–
	CT-wet-BCR	25	6.02	0.08	NS	0.6
	CT-wet-DSR	7	6.84	0.90	0.0005	18.5
	CT-dry-BCR	4	6.04	0.17	NS	0.8
	CT-dry-DSR	6	6.07	0.23	NS	4.4
Thailand	CT-TPR	24	3.63	–	–	–
	CT-wet-seeding (CT-wet-BCR, CT-wet-DSR)	16	3.73	0.24	NS	9
	CT-dry-seeding (CT-dry-BCR, CT-dry-DSR)	9	3.83	−0.07	NS	2.5

Table 7 (*Continued*)

Country	Tillage and CE methods[a]	N^b	Adjusted mean yield (t ha^{-1})[c]	Δ Yield (t ha^{-1})[d]	*P* value[e]	% Change
	ZT-dry-seeding (ZT-dry-BCR, ZT-dry-DSW)	4	3.61	−0.20	NS	−3.4

NS, nonsignificant.
[a] Refer to Table 5 for a description of tillage and CE methods.
[b] Number of studies.
[c] Adjusted mean yield calculated using SAS mixed model analysis.
[d] Adjusted mean of change in yield over CT-TPR calculated using SAS mixed model analysis.
[e] Based on analysis of change (Δ yield) data (pair comparison with CT-TPR).

It is also important to note that the performance of DSR can also vary from location to location within a country. For example, in the northwestern IGP, there is a tendency of a yield penalty with Dry-DSR (Gathala *et al.*, 2011; Jat *et al.*, 2009; Saharawat *et al.*, 2009) but not in the eastern IGP (Singh *et al.*, 2009c). A possible reason for this differential performance in northwestern versus eastern IGP is lower rainfall in the former (400–750 mm $year^{-1}$) than in the latter (1000–1500 mm $year^{-1}$) (Gupta and Seth 2007). Flooding of rice after successful establishment can alleviate nutrient deficiencies (i.e., Fe and Zn) and soil-borne diseases (i.e., nematodes). Also in the eastern IGP, current yields of CT-TPR are much lower than that in the northwestern IGP; therefore, it is easier to achieve equivalent yield with DSR.

The causes of lower yield in Wet- and Dry-DSR reported by researchers in different production zones may include (1) uneven or poor CE (Rickman *et al.*, 2001), (2) inadequate weed control (Johnson and Mortimer, 2005; Kumar *et al.*, 2008a; Rao *et al.*, 2007; Singh *et al.*, 2005), (3) higher spikelet sterility than in puddled transplanting (Bhushan *et al.*, 2007; Choudhury *et al.*, 2007), (4) higher crop lodging, especially in wet seeding and broadcasting (Fukai, 2002; Ho and Romli, 2002; Rickman *et al.*, 2001; Yoshinaga, 2005), and (5) insufficient knowledge of water and nutrient management (micronutrient deficiencies) (Choudhury *et al.*, 2007; Humphreys *et al.*, 2010; Sharma *et al.*, 2002; Singh *et al.*, 2002a; Yadvinder-Singh *et al.*, 2008; Sudhir-Yadav *et al.*, 2011a,b).

In studies in which these constraints have been addressed, equivalent or higher yields are often reported under DSR than in CT-TPR (Bhushan *et al.*, 2007; San-oh *et al.*, 2004; Tabbal *et al.*, 2002; Yoshinaga *et al.*, 2001). Technologies have been developed or progress has been made to overcome some of the constraints in DSR. For example, (1) coating of pregerminated seeds with calcium peroxide to facilitate seedling establishment in anaerobic conditions in wet seeding or water seeding (Ota and Nakayama, 1970), (2) the development

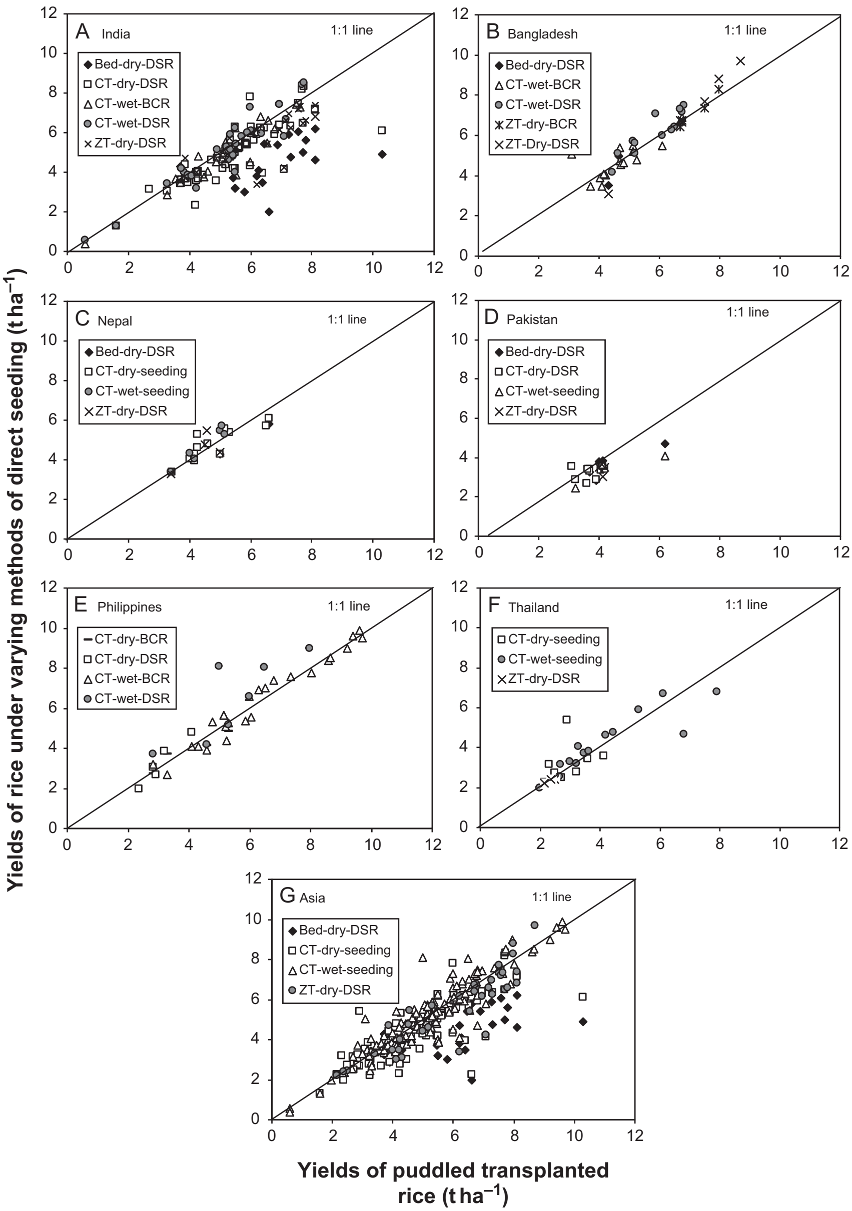


Figure 7 Rice grain yield of puddled transplanted rice versus alternative tillage and rice establishment (CE) methods from researcher-managed on-station and on-farm trials: (A) India, (B) Bangladesh, (C) Nepal, (D) Pakistan, (E) Philippines, (F) Thailand, and (G) Asia (overall of all six countries). See Table 5 for details of tillage and CE methods.

of new-generation precise seeding and land-leveling machinery for dry drill seeding (Gopal *et al.*, 2010; Gupta *et al.*, 2006; Rickman, 2002), (3) integrated weed management (IWM), including the use of effective herbicides and nonchemical methods for weed control (Chauhan and Johnson, 2010; Rao *et al.*, 2007; Singh *et al.*, 2009b), and (4) breeding more lodging-tolerant genotypes and using of hill seeding or row seeding instead of broadcasting to minimize lodging (Yoshinaga, 2005).

5.2. Irrigation water application and irrigation water productivity

A review of 44 studies (36 researchers-managed and 8 farmers-managed) from different countries showed 12–33% (139–474 mm) lower irrigation water use in DSR than in flooded CT-TPR (Table 8). The reduction in irrigation water use varied with type of DSR method, ranging from 139 mm (12%) in wet seeding on puddled soil (CT-wet-seeding) to 304–385 mm (21–25%) in dry seeding after tillage (CT-dry-seeding) or zero tillage (ZT-dry-seeding), and 474 mm (33%) in dry seeding on raised beds (Bed-dry-DSR).

Table 8 Analysis of irrigation water application comparisons between puddled transplanting and various alternative tillage and crop establishment methods

Tillage and CE methods[a]	*N*[b]	Adjusted mean of irrigation water use (mm)[c]	Change from CT-TPR (mm)[d]	*P* value[e]	% Change
CT-TPR	44	1372	–	–	–
CT-wet-seeding (CT-wet-BCR, CT-wet-DSR)	27	1234	−139	0.0307	−12
CT-dry-seeding (CT-dry-BCR, CT-dry-DSR)	31	1074	−304	0.001	−21
Bed-dry-DSR	14	887.5	−474	0.001	−33
ZT-dry-seeding (ZT-dry-BCR, ZT-dry-DSR)	6	1039	−385	0.001	−25

[a] Refer to Table 5 for a description of tillage and CE methods.
[b] Number of studies.
[c] Adjusted mean of irrigation water calculated using SAS mixed model analysis.
[d] Adjusted mean of change in irrigation water application over CT-TPR calculated using SAS mixed model analysis.
[e] Based on analysis of change data (pair comparison with CT-TPR).

The relatively lower water use in Wet-DSR than in CT-TPR despite its longer main field duration may be because of fewer continuous flooded days in the main field (Fig. 8A and B). In CT-TPR, the field is generally kept continuously flooded (Fig. 8A). Whereas in Wet-DSR, during the first 10 days, very little or no irrigation is applied and then irrigation is either applied at 2- to 3-day intervals or relatively shallow flooding is maintained during the early part of vegetative growth to avoid submergence of young seedlings, thereby reducing seepage, percolation, and evaporation losses. Moreover, the Wet-DSR crop is harvested about 10–15 days earlier than CT-TPR; therefore, total duration from seed to seed is reduced in this method (Fig. 8B). Another reason reported for lower water use in Wet-DSR is the shorter land preparation period than in CT-TPR. In some areas, for example, in the largest surface irrigation scheme in Central Luzon, called UPRIIS (Upper Pampanga River Integrated Irrigation System), because of a lack of tertiary field channels, the whole main field is soaked when the nursery is prepared and kept flooded during the entire duration of the nursery for CT-TPR. This results in a longer land preparation period and higher seepage, percolation, and evaporation losses (Tabbal *et al.*, 2002). In Wet-DSR, the main field is soaked, and the land is prepared 2–3 days prior to sowing. In Dry-DSR, lower water use than that in CT-TPR may be attributed to savings in water used for puddling in CT-TPR and the AWD irrigation method instead of continuous flooding in CT-TPR (Fig. 8C).

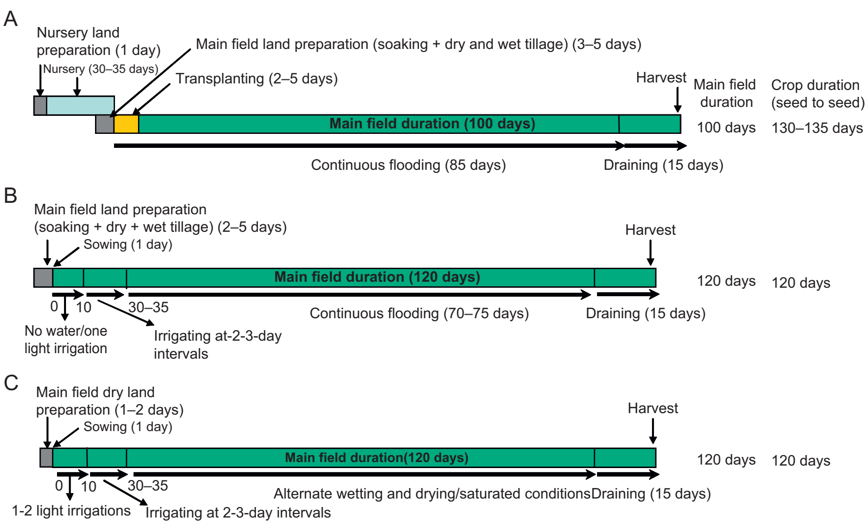


Figure 8 Various cultural activities, including irrigation schedules of puddled transplanting (A), direct wet seeding (B), and direct dry seeding (C). Modified from Tabbal *et al*. (2002).

Although the overall trend is a savings in irrigation water application with alternative tillage and methods of rice establishment, some authors have reported higher irrigation water use (Bhuiyan *et al.*, 1995; Hukkeri and Sharma, 1980), which could be due to (1) a longer crop growth period in the main field in DSR (Wet- and Dry-DSR) than in CT-TPR (Rashid *et al.*, 2009; Fig. 8), and (2) higher percolation losses, especially with Dry-DSR (Sudhir-Yadav *et al.*, 2011a,b). Rainfall pattern and time of occurrence are another major deciding factor in irrigation water use and resulting savings (Bhushan *et al.*, 2007; Saharawat *et al.*, 2010). If the onset of rain coincides with puddling and extends for a few days after CE, then irrigation water use declines drastically. Bhushan *et al.* (2007) and Gathala *et al.* (2011) highlighted savings in irrigation water use in years with favorable and unfavorable rainfalls. There is a trade-off between savings of irrigation water during land preparation and increased water use during crop growth, which is highly influenced by rainfall pattern.

Although all the DSR methods (wet or dry) were effective in saving irrigation water, their water use productivity (grain yield per liter of water applied) was higher only for wet seeding (CT-wet-seeding) and dry seeding on tilled soil (CT/RT-dry-seeding) (Fig. 9). However, irrigation water productivity in dry seeding on raised beds (Bed-dry-DSR) and zero-till (ZT-dry-DSR) was similar to that of CT-TPR due to lower yields in these systems. Bouman and Tuong (2001) also observed that most of the water-saving technologies, including DSR, result in some yield losses. Therefore, water productivity is a better indicator for making a comparison of different technologies in terms of their effective use of irrigation water and food production (Molden, 1997; Tuong, 1999). The results suggest that, to have a significant impact on irrigation water savings, yields of Bed-dry-DSR and ZT-dry-DSR should be further improved. There is also an urgent need to

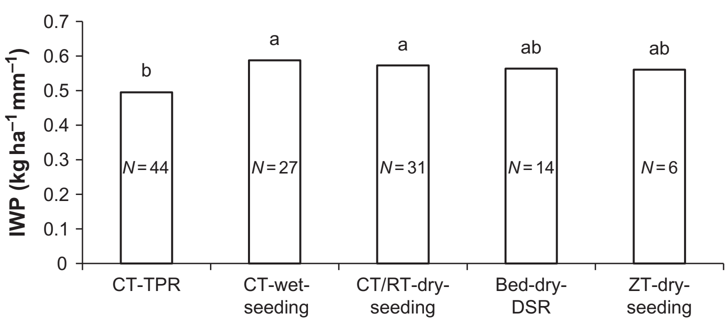


Figure 9 Irrigation water productivity (IWP) of major tillage and crop establishment methods in rice. See Table 5 for treatment details. *N* is the number of studies. Values followed by the same letter are not significantly different from each other at $P < 0.05$ by the Tukey test.

develop efficient irrigation schedules for the selected alternative tillage and rice establishment methods such as Dry-DSR. Irrigation scheduling should take various components of water and nutrient balance, and weed dynamics, into consideration.

5.3. Labor use

Compared with CT-TPR, DSR is a labor-saving technology. Large variations in total labor requirement for various field operations for diverse practices were reported (Table 9), which may largely be due to differences in the level of mechanization used. Depending on the method of land preparation and CE, the labor requirement in DSR can be up to 60% lower, with an average savings of 27% compared with CT-TPR. In Wet-DSR, labor savings ranged from none to 46%, with an average of 25%, whereas in Dry-DSR, savings ranged from 4% to 60%, with an average of 29%. The variation reported by different studies in labor savings primarily depends on labor used in weed control. Labor use is higher (12–200%) for controlling weeds in DSR than in CT-TPR. If weeds are controlled effectively with herbicides, the labor savings can be substantial.

Direct seeding (both wet and dry) avoids nursery raising, seedling uprooting, and transplanting, and thus reduces the labor requirement. Dry-DSR also avoids puddling operations, and thus further saves labor use. Since land preparation is mostly mechanized, there is more savings in machine labor than in human labor in this operation. Short- to medium-term on-station studies reported 34–46% savings in machine labor requirement in ZT-dry-DSR compared with CT-TPR (Bhushan *et al.*, 2007; Saharawat *et al.*, 2010).

In addition to labor savings, the demand for labor is spread out over a longer period in DSR than in transplanted rice. Conventional practice (CT-TPR) requires much labor in the critical operation of transplanting, which often results in a shortage of labor. The spread-out labor requirement helps in making full use of family labor and having less dependence on hired labor.

5.4. Economics

A major reason for farmers' interest in DSR is the rising cost of cultivation and decreasing profits with conventional practice (CT-TPR). Farmers likely prefer a technology that gives higher profit despite similar or slightly lower yield. Overall analysis of 77 published studies shows that various methods of direct seeding reduced the cost of production by US$9–125 ha^{-1} compared with conventional practice (CT-TPR) (Table 10). The largest reductions in cost occurred in practices in which reduced or zero tillage was combined with Dry-DSR. These cost reductions were largely due to either reduced labor cost or tillage cost or both under DSR

Table 9 Labor use (person-days ha^{-1}) in different field operations for direct-seeded and transplanted rice

			Labor use (person-days ha^{-1})								
S. no.	Country	Tillage and CE method[a]	Nursery	Seedling uprooting	Land preparation	Crop establishment (sowing/transplanting)	Weeding	Harvesting/ threshing	Total	% saving	Reference
1	Bangladesh	CT-TPR	14	11	5	28	34	39	139	0	Rahman *et al.* (2008)
		CT-wet-BCR	0	0	6	2	54	50	121	13	
2	Bangladesh	CT-TPR	2	10	–[b]	27	34	32	118	0	Rashid *et al.* (2009)
		CT-wet-DSR (drum)	0	0	–	1	38	36	92	23	
3	India	CT-TPR	24[c]		–	22	20	–	109	0	Ramasamy *et al.* (2006)
		CT-wet-BCR	0[c]b		–	1	25	–	69	37	
4	India	CT-TPR	5	–	–	75	30	–	229	0	Thakur *et al.* (2004)
		CT-wet-BCR	0	–	–	3	90	–	186	19	
		CT-wet-DSR (line sown)	0	–	–	20	40	–	214	7	
		CT-dry-BCR	0	–	–	2	100	–	220	4	
5	India	CT-TPR	–	–	–	–	–	–	66	0	Bhushan *et al.* (2007)
		Bed-dry-DSR	–	–	–	–	–	–	47	29	
		ZT-dry-DSR	–	–	–	–	–	–	47	28	
6	India	CT-TPR	–	–	–	–	–	–	64	0	Saharawat *et al.* (2010)
		CT-wet-SR (drum)	–	–	–	–	–	–	67	0	
		ZT-dry-DSR	–	–	–	–	–	–	56	13	
7	Korea	CT-TPR (machine)	–	–	–	-	–	–	42	0	Lee *et al.* (2002)
		CT-wet-seeding (CT-wet-BCR, CT-wet-DSR)	–	–	–	–	–	–	31	27	
		CT-dry-seeding	–	–	–	–	–	–	29	31	
8	Malaysia	CT-TPR	–	–	–	–	–	–	237	0	Wong and Morooka (1996)
		CT-wet-BCR	–	–	–	–	–	–	80	66	
9	Philippines	CT-TPR	–	–	–	–	–	–	53		Pandey and Velasco (1998)
		CT-wet-seeding (CT-wet-BCR, CR-wet-DSR)	–	–	–	–	–	–	30	40	
		CT-dry-seeding (CT-dry-BCR, CT-dry-DSR)	–	–	–	–	–	–	40	20	

(*Continued*)

Table 9 (*Continued*)

S. no.	Country	Tillage and CE method[a]	Labor use (person-days ha^{-1})								Reference
			Nursery	Seedling uprooting	Land preparation	Crop establishment (sowing/transplanting)	Weeding	Harvesting/ threshing	Total	% saving	
10	Philippines	CT-TPR	3	4	10	22	–	–	97	0	Tisch and Paris (1994)
		CT-wet-BCR	0	0	10	2	–	–	49	49	
11	Philippines	CT-TPR	–	–	–	–	–	–	49	0	Pandey *et al.* (1995)
		CT-dry-seeding (CT-dry-BCR, CT-dry-DSR)	–	–	–	–	–	–	22	60	
12	Thailand	CT-TPR	2	–	6	23	3	29	65	0	Sumita and Ando (2001)
		CT-dry-BCR	0	–	4	3	1	28	40	39	
13	Thailand	CT-TPR	–	–	4	15	–	–	39	0	Isvilanonda (2002)
		CT-wet-BCR	–	–	3	2	–	–	30	24	
14	Thailand	CT-TPR	–	–	–	–	–	–	74	0	Pandey *et al.* (2002)
		CT-wet-BCR	–	–	–	–	–	–	40	46	
15	Vietnam	CT-TPR	–	–	–	–	–	–	68	0	Pandey *et al.* (2002)
		CT-wet-seeding (CT-wet-BCR, CT-wet-DSR)	–	–	–	–	–	–	38	40	
		CT-dry-seeding (CT-dry-BCR, CT-dry-DSR)	–	–	–	–	–	–	38	40	

[a] Refer to Table 5 for a description of tillage and CE methods.
[b] Data not available.
[c] This is sum of labor used in nursery raising and seedling uprooting.

Table 10 Analysis of cost of production and net income comparisons between puddled transplanting and various alternative tillage and crop establishment methods in Asia

Country	Tillage and CE method[a]	N[b]	Total cost (US$ ha^{-1})[c]	Δ cost (US $ ha^{-1})[d]	*P* value[e]	N[c]	Net income (US$ ha^{-1})[f]	Δ NI (US $ ha^{-1})[g]	*P* value[h]
India	CT-TPR	15	397	–	–	35	277	–	–
	CT-wet-BCR	3	365	−26	NS	7	265	−6	NS
	CT-wet-DSR	3	357	−31	NS	17	322	35	0.1100
	CT-dry-DSR	13	352	−48	0.0143	21	281	8	NS
	Bed-dry-DSR	3	283	−120	0.0002	7	130	−166	<0.0001
	ZT-dry-DSR	3	277	−125	0.0002	8	308	1	NS
Bangladesh	CT-TPR	10	409	–	–	11	475	–	–
	CT-wet-BCR	3	395	−13.0	NS	5	493	27	0.070
	CT-wet-DSR	5	390	−9.0	NS	6	552	74	0.0001
	ZT-dry-BCR	3	380	−44.0	0.0006	4	495	13	NS
	ZT-dry-DSR	4	384	−40.0	0.0013	4	535	52	0.0033
Nepal	CT-TPR	5	212	–	–	5	419	–	–
	CT-wet-seeding (CT-wet-BCR, CT-wet-DSR)	5	180	−34	0.0927	3	500	89	0.0722
	CT/RT-dry-DSR-Flat	3	176	−39	0.0361	5	496	81	0.0436
Philippines	CT-TPR	12	408	–	–	12	362	–	–
	CT-wet-seeding (CT-wet-BCR, CT-wet-DSR)	9	429	8	NS	9	498	132	0.0022
	CT-dry-seeding (CT-dry-BCR, CT-dry-DSR)	7	380	−25	0.0532	7	449	94	0.0218
Thailand	CT-TPR	12	288	–	–	12	60	–	–
	CT-wet-seeding (CT-wet-BCR, CT-wet-DSR)	12	256	−32	0.0004	12	117	58	0.0043

(*Continued*)

Table 10 (*Continued*)

Country	Tillage and CE method[a]	N[b]	Total cost (US$ ha^{-1})[c]	Δ cost (US $ ha^{-1})[d]	*P* value[e]	*N*[c]	Net income (US$ ha^{-1})[f]	Δ NI (US $ ha^{-1})[g]	*P* value[h]
Asia	CT-TPR	57	359	–	–	77	286	–	–
	CT-wet-seeding (CT-wet-BCR, CT-wet-DSR)	40	338	−22	0.0259	61	338	51	0.0115
	CT/RT-dry-seeding (CT/RT-dry-BCR, CT/RT-dry-DSR)	28	324	−29	0.0084	36	314	30	0.0619
	Bed-dry-DSR	8	301	−58	0.0002	12	221	−62	0.0003
	ZT-dry-seeding (ZT-dry-BCR, ZT-dry-DSR)	11	294	−80	<0.0001	18	337	51	0.0197

[a] Refer to Table 5 for a description of tillage and CE methods.
[b] Number of studies.
[c] Adjusted mean of production cost calculated using SAS mixed model analysis.
[d] Adjusted mean of change in production cost over CT-TPR calculated using SAS mixed model analysis.
[e] Based on analysis of change data (Δ cost) (pair comparison with CT-TPR).
[f] Adjusted mean of net income calculated using SAS mixed model analysis.
[g] Adjusted change in net income over CT-TPR using SAS mixed model analysis.
[h] Based on analysis of change data (Δ net income) (pair comparison with CT-TPR).

systems. In regions where wages are high (e.g., Haryana and Punjab states of India), the labor cost savings in rice establishment can reach US$50 ha^{-1} (Kumar *et al.*, 2009).

However, these reduced costs did not always translate into increased profitability. For example, the cost of growing rice on raised beds in India was the lowest among different alternative tillage and CE methods but there was a net loss of returns of US$166 ha^{-1} compared with CT-TPR, which was primarily due to associated lower grain yield. Increases in net returns in other direct-seeding methods compared to CT-TPR were highly variable, ranging from US$1 to 132 ha^{-1} primarily because of large yield variability. On average, the increases in net returns with direct-seeding on puddled or zero-till soil were similar (US$51 ha^{-1}). Overall, all types of direct-seeding methods, except Bed-dry-DSR, were either more profitable than or equally profitable as puddled transplanted rice.

The labor and water costs are likely to increase in future which will make DSR economically more attractive to the farmers.

5.5. Greenhouse gas (GHG) emissions

Agricultural practices play an important role in the emissions of carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O)—three important GHGs that contribute to global warming. Agriculture's share in the total emissions of N_2O, CH_4, and CO_2 are 60%, 39%, and 1%, respectively (OECD, 2000), with rice-based cropping systems playing a major role. Rice production systems impact global warming potential (GWP) primarily through effects on methane, but N_2O and CO_2 effects can also be important in some systems. The GWP of CH_4 and N_2O is 25 and 298 times higher than that of CO_2 (IPCC, 2007). GHG emissions, especially CO_2 and CH_4 from rice fields, are large and very sensitive to management practices. Therefore, rice is an important target for mitigating GHG emissions (Wassmann *et al.*, 2004). Flooded rice culture with puddling and transplanting is considered one of the major sources of CH_4 emissions because of prolonged flooding resulting in an anaerobic soil condition. It accounts for 10–20% (50–100 Tg $year^{-1}$) of total global annual CH_4 emissions (Houghton *et al.*, 1996; Reiner and Milkha, 2000).

Studies comparing CH_4 emissions from different tillage and CE methods but with similar water management (continuous flooding/mid-season drainage/intermittent irrigation) in rice revealed that, except in one study (Setyanto *et al.*, 2000), CH_4 emissions were lower with Wet- or Dry-DSR than with CT-TPR (Table 11). The reported reduction in CH_4 emissions was higher in Dry-DSR than in Wet-DSR. Under continuous flooding, the reduction in CH_4 emissions ranged from 24% to 79% in Dry-DSR and from 8% to 22% in Wet-DSR, whereas, under intermittent irrigation, the reduction ranged from 43% to 75% in Dry-DSR compared with CT-TPR. However, when DSR was combined with mid-season drainage or

Table 11 Effects of various tillage and crop establishment methods on methane emissions in Asia

S. no.	Location/ country	Year/season	Tillage and crop establishment method	Water management	Seasonal total emission (kg CH_4 ha^{-1})	% change from TPR or puddling	Yield (t ha^{-1})	Reference
1	Beijing	1991	CT-TPR	Intermittent irrigation	299	0	4.5	Wang *et al.* (1999)
			CT-dry-seeding	Intermittent irrigation	74	−75	3.6	
2	Southeastern Korea	1996	CT-TPR (30-day-old seedling)	Continuous flooding	403	0	5.3	Ko and Kang (2000)
			CT-TPR (8-day-old seeding)	Continuous flooding	424	5	5.4	
			CT-wet-seeding	Continuous flooding	371	−8	5.4	
			CT-dry-seeding	Continuous flooding	269	−33	5.3	
3	Milyang, Korea	1998–2000	CT-TPR	Continuous flooding	402	0	–	Ko *et al.* (2002)
			CT-dry-seeding	Continuous flooding	241	−40	-	
			ZT-dry-TPR	Continuous flooding	295	−27		
			ZT-dry-seeding	Continuous flooding	258	−36		

4	Jakenan, Indonesia	1993 WS	CT-TPR	Continuous flooding	229	0	4.7	Setyanto *et al.* (2000)
			CT-wet-seeding	Continuous flooding	256	12	7.1	
			CT-TPR	Rainfed	59	0	4.9	Setyanto *et al.* (2000)
			CT-dry-seeding	Rainfed	26	−56	4.4	
5	Akasaka, Japan	1992–1994	CT-TPR	Continuous flooding	159	0	–	Ishibashi *et al.* (2001)
			ZT-dry-seeding	Continuous flooding	34	−79	–	
	Suimon, Japan	1994–1997	CT-TPR	Continuous flooding	271	0	–	Ishibashi *et al.* (2001)
			ZT-dry-seeding	Continuous flooding	129	−52	–	
6	Sanyoh, Japan	1992-2000	TPR	Continuous flooding	330	0	–	Tsuruta (2002)
			ZT-dry-DSR	Continuous flooding	252	−24	–	
7	Hachirogata polder, Japan	2004–2005	CT-TPR	Intermittent irrigation	179	0	5.4	Harada *et al.* (2007)
			Unpuddled (CT-dry)-TPR	Intermittent irrigation	182	2 ns	5.6	
			ZT-dry-TPR[a]	Intermittent irrigation	102	−43	5.5	

(*Continued*)

Table 11 (*Continued*)

S. no.	Location/ country	Year/season	Tillage and crop establishment method	Water management	Seasonal total emission (kg CH_4 ha^{-1})	% change from TPR or puddling	Yield (t ha^{-1})	Reference
8	Maligaya, Philippines	1997 DS	CT-TPR	Continuous flooding	89	0	7.9	Corton *et al.* (2000)
			CT-wet-DSR	Continuous flooding	75	−16	6.7	
			CT-TPR	Midseason drainage	51	0	7.7	Corton *et al.* (2000)
			CT-wet-DSR	Midseason drainage	48	−6	6.4	
		1997 WS	CT-TPR	Continuous flooding	348	0	5.4	Corton *et al.* (2000)
			CT-wet-DSR	Continuous flooding	272	−22	3.5	
			CT-TPR	Midseason drainage	323	0	5.5	Corton *et al.* (2000)
			CT-wet-DSR	Midseason drainage	150	−54	3.4	
		1998 DS	CT-TPR	Continuous flooding	90	0	8.5	Corton *et al.* (2000)
			CT-wet-DSR	Midseason drainage	16	−82	7.7	
			CT-wet-DSR	Intermittent irrigation	7	−92	7.1	

9	Pantnagar, India	2004	CT-TPR	–	315	0	6.8	Singh *et al.* (2009a)
			CT-dry-DSR	–	220	−30	6.6	
10	Karnal, India[b]	2006–2007	CT-TPR	–	59	0	–	Saharawat (unpublished)
			CT-dry-DSR	–	25	−58	–	
11	Modipuram, India[b]	2000–2005	CT-TPR	–	60	0	–	Pathak *et al.* (2009)
			CT-dry-DSR	–	25	−58	–	

Refer to Table 5 for a description of tillage and CE methods.

[a] In all treatments (zero tillage, no puddling, and puddling), rice was transplanted.

[b] Values are based on simulation modeling.

intermittent irrigation, the reduction in CH_4 emissions increased further compared with flooded CT-TPR. For example, in Wet-DSR, the reduction in CH_4 increased from 16%–22% (under continuous flooding) to 82%–92% (under mid-season drainage or intermittent irrigation) compared with CT-TPR under continuous flooding (Corton *et al.*, 2000; Table 11). Wassmann *et al.* (2004) also suggested that CH_4 mitigation effects can be further enhanced if Wet- or Dry-DSR is combined with mid-season drainage.

CH_4 emissions even in CT-TPR vary considerably from study to study (Table 11). This difference could be because of individual or combined effects of different soil characteristics, climatic conditions, and management such as soil pH, redox potential, soil texture, soil salinity, temperature, rainfall, and water management (Aulakh *et al.*, 2001). The reason for low CH_4 emissions from Dry-DSR is aerobic conditions, especially during the early growth stage. Even under Wet-DSR, field is kept aerobic until seedlings are established. Anaerobic conditions are a prerequisite for the activities of methanogenic bacteria and CH_4 production. Methane emission starts at redox potential of soil below -150 mV and is stimulated at less than -200 mV (Jugsujinda *et al.*, 1996; Masscheleyn *et al.*, 1993; Wang *et al.*, 1993).

Although water-saving technologies including Dry-DSR can reduce CH_4 emissions, relatively more soil aerobic states can also increase N_2O emissions. Nitrous oxide production increases at redox potentials above 250 mV (Hou *et al.*, 2000). In a study conducted in India comparing N_2O emissions from CT-TPR and different Dry-DSR methods (CT-dry-DSR, Bed-dry-DSR, ZT-dry-DSR), it was found that N_2O emissions were 0.31–0.39 kg N ha^{-1} in CT-TPR, which increased to 0.90–1.1 kg N ha^{-1} in CT-dry-DSR and Bed-dry-DSR and 1.3–2.2 kg N ha^{-1} in ZT-dry-DSR (Fig. 10). Similarly, a study conducted by Ishibashi *et al.* (2007) in western Japan also observed higher emissions of N_2O under ZT-dry-DSR than in CT-TPR. These results suggest the need to deploy strategies to reduce N_2O emissions from Dry-DSR for minimizing adverse impacts on the environment. Hou *et al.* (2000) suggested developing water management practices in such a way that soil redox potential can be kept at intermediate range (-100 to $+200$ mV) to minimize emissions of both CH_4 and N_2O. This range is high enough to prevent CH_4 production and low enough to encourage N_2O reduction to N_2 as the critical soil redox potential identified for N_2O production is $+250$ mV (Hou *et al.*, 2000).

An overall effect of direct-seeding methods on GWP depends on total emissions of all three major GHGs. It has been observed that measures to reduce one source of GHG emissions often lead to increases in other GHG emissions, and this trade-off between CH_4 and N_2O is a major hurdle in devising an effective GHG mitigation strategy for rice (Wassmann *et al.*, 2004). Very few studies have compared different rice production systems in

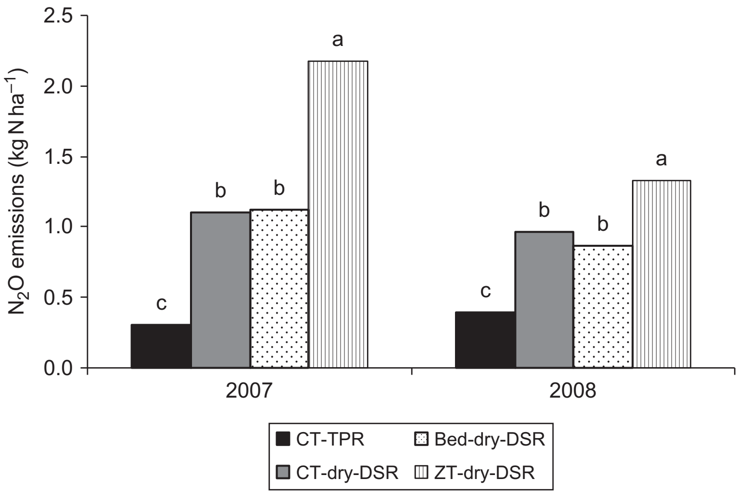


Figure 10 Nitrous oxide emission from puddled transplanted rice and methods of alternate tillage and crop establishment in 2007 and 2008 at Modipuram in India. Within years, means with the same letters are not significantly different at the 0.05 level by the Tukey test. See Table 5 for details of tillage and crop establishment methods. Source: Sharma *et al.* (unpublished).

terms of total GWP taking into account all three GHGs. Ishibashi *et al.* (2009) compared ZT-dry-DSR with CT-TPR and found ZT-dry-DSR 20% more efficient in reducing GWP. Pathak *et al.* (2009) simulated for Indian conditions and found that Dry-DSR on raised beds or ZT has potential to reduce CO_2 equivalent ha^{-1} by 40–44% compared with CT-TPR. Harada *et al.* (2007) reported that, just by changing puddling to zero tillage, GWP declined by 42% in Japan.

In summary, despite relatively higher emissions of N_2O in Dry-DSR, GWP of Dry-DSR tends to be lower than for flooded CT-TPR because of substantially higher emissions of CH_4 in CT-TPR. However, more systematic studies involving simultaneous measurements of three GHGs are needed to come to sound conclusions. Further, considering the burgeoning global demand for food, fiber, and fuel, appropriate GHG emission strategies must involve ecologically intensive crop management practices that enhance nutrient use efficiency and maintain high yields (Cassman, 1999).

6. Potential Benefits and Risks Associated with Direct-Seeded Rice

Direct-seeding of rice has the potential to provide several benefits to farmers and the environment over conventional practices of puddling and transplanting. However, it is also important to understand and predict

Table 12 Benefits and risks/limitations associated with direct seeding of rice

A. Benefit
1. Labor savings ranged from 0% to 46%, with an average of 25% in wet direct seeding and 4% to 60%, with an average of 29%, in dry direct seeding
2. Reduces drudgery by eliminating transplanting operation
3. Water savings ranged from 12% to 35% depending on type of DSR. Water savings in different types of DSR ranked in the following order: CT-wet-seeding < CT-dry-seeding = ZT-dry-DSR < Bed-dry-DSR
4. Reduces irrigation water loss through percolation due to fewer soil cracks
5. Reduces methane emissions (6–92% depending on types of DSR and water management)
6. Reduces cost of cultivation, ranging from 2% to 16% (US\$8–34 ha^{-1}) in wet DSR and from 6% to 32% (US\$29-125 ha^{-1}) in Dry-DSR
7. Increases the total income of farmers (US\$30–51 ha^{-1} depending on type of DSR)
8. Allows timely planting of subsequent crop due to early harvest of direct-seeded rice crop by 7–14 days
B. Risk
1. Sudden rain immediately after seeding can adversely affect crop establishment
2. Reduces availability of soil nutrients such as N, Fe, and Zn especially in Dry-DSR
3. Appearance of new weeds such as weedy or red rice
4. Increases dependence on herbicides
5. Increases incidence of new soil-borne pests and diseases such as nematodes
6. Enhances nitrous oxide emissions from soil
7. Relatively more soil C loss due to frequent wetting and drying

possible risks or threats that direct seeding may have in the long run. Table 12 summarizes these benefits and risks.

7. Weeds in Direct-Seeded Rice: A Major Constraint

Weeds are a major constraint to the success of DSR in general and to Dry-DSR in particular (Johnson and Mortimer, 2005; Rao *et al.*, 2007; Singh *et al.*, 2006). Research has shown that, in the absence of effective weed control options, yield losses are greater in DSR than in transplanted rice (Baltazar and De Datta, 1992; Rao *et al.* 2007). Weeds are more problematic in DSR than in puddled transplanting because (1) emerging DSR seedlings are less competitive with concurrently emerging weeds and (2) the initial flush of weeds is not controlled by flooding in Wet- and Dry-DSR (Kumar *et al.*, 2008a; Rao *et al.*, 2007).

It is important to review the weed-related issues emerging with the adoption of DSR based on the experiences from those countries where transplanting is being replaced widely by DSR. This would assist in developing effective and economically viable medium- to long-term sustainable weed control strategies. This section reviews some of the weed related issues that have emerged in countries where DSR is widely practiced.

7.1. Evolution of weedy rice

Weedy rice (*O. sativa* f. *spontanea*), also known as red rice, has emerged as a serious threat to rice production in areas where transplanting is widely replaced by direct seeding, especially in many Asian countries. Table 13 describes the evolution of weedy rice and its key characteristics in relation to the adoption of DSR.

Weedy rice is highly competitive and causes severe rice yield losses ranging from 15% to 100% (Table 13). Weedy rice densities of 4, 16, and 25 m^{-2} reduced rice yield by 13%, 37%, and 48%, respectively (Pantone and Baker, 1991). Other studies reported up to a 58% reduction in rice yield at a density of 40 weeds m^{-2} (Eleftherohorinos *et al.*, 2002) and up to 82% at a density of 215 weeds m^{-2} (Diarra *et al.*, 1985). Smith (1988) reported a density of 1–3 plants m^{-2} as the threshold level for control to avoid yield loss. Weedy rice also reduces milling quality if it gets mixed with rice seeds during harvesting (Ottis *et al.*, 2005).

Weedy rice is difficult to control because of its genetic, morphological, and phenological similarities with rice. Selective control of weedy rice was never achieved at a satisfactory level with herbicides (Noldin *et al.*, 1999a,b). In Malaysia, proper land preparation coupled with the stale seedbed technique using nonselective herbicides (paraquat/glyphosate/glufosinate) before planting rice has been recommended to reduce the density of weedy rice (Karim *et al.*, 2004). FAO (1999) recommends an integrated approach that combines preventive, cultural, and chemical methods. The keys for control and to avoid further infestation are to use clean and certified seeds (Rao *et al.*, 2007). Azmi and Abdullah (1998) observed that preplant application of soil-incorporated molinate at 4.5 kg ai ha^{-1} was effective in reducing the seed bank of weedy rice. Herbicide-resistant rice technologies offer opportunities for selective control of weedy rice but the risk of gene flow from herbicide-resistant rice to weedy rice poses a constraint for the long-term utility of this technology (Kumar *et al.*, 2008b).

Weedy/red rice could become a major threat to rice production where Dry-DSR replaces CT-TPR. Therefore, there is a need to develop preventive management strategies to deal with the weedy-rice problem in Dry-DSR.

Table 13 Emergence of weedy rice in Asian countries where direct seeding is predominant

Country	DSR introduction year	DSR area (% of total rice area)	Weedy rice first detection year	Rice grain yield losses due to weedy rice	Current status of weedy rice
Korea	1991[a]	In 1995, it was 11% and decreased to 4.5% in 2007[b]	NA	NA	Major constraint with up to 35% infestation in mostly Dry-DSR[b]
Malaysia	Late 1970s or early 1980s[c]	>95[d]	1988[e]	Up to 74% in heavily infested areas[f,g]	Serious infestation of weedy rice observed after 20 consecutive seasons of direct seeding.[h] In Muda area, almost all fields infested with weedy rice[i]; 10% is heavily infested.[j] A similar situation is also reported in other Malaysian irrigation schemes[i,k]

Sri Lanka	NA	> 93[l]	1992[l]	30–100%[m]	In 2008, in Ampara and Puttalam districts, many farmers could not cultivate their fields because of weedy rice[l]
Thailand	1980s	34[n]	2001[o]	60–80%[p]	First detected at two locations in central Plain and now found in seven provinces of central and lower northern Thailand in about 3.0 million ha of area of direct-seeded rice[b]

(*Continued*)

Table 13 (*Continued*)

Country	DSR introduction year	DSR area (% of total rice area)	Weedy rice first detection year	Rice grain yield losses due to weedy rice	Current status of weedy rice
Vietnam	Early 1980s[q]	39–47[n]	1994[r]	15–70%[s]	Weedy rice is a major problem in Mekong Delta area in summer–autumn crops. In wet seeding, it is not a major problem[s,t]

NA, data not available.

[a] Kim and Ha (2005).
[b] Gressel and Valverde (2009).
[c] Azmi *et al.* (2005).
[d] Azmi (personal communication).
[e] Azmi and Abdullah (1998).
[f] Watanabe *et al.* (1996).
[g] Bakar *et al.* (2000).
[h] Ho (1996).
[i] Begum *et al.* (2005).
[j] Azmi and Baki (2007).
[k] Mispan and Baki (2008).
[l] Weerakoon *et al.* (2011).
[m] Gunawardana (2008).
[n] Pandey and Velasco (2002).
[o] Maneechote *et al.* (2004).
[p] Vongsaroj (2000).
[q] Can and Xuan (2002).
[r] Chin (1997).
[s] Chin (2001).
[t] Mai *et al.* (2000).

7.2. Changes in composition and diversity of weed flora and a shift toward more difficult-to-control weeds

Changes in rice establishment method as well as water, tillage, and weed management practices in DSR lead to changes in weed composition and diversity. Weed flora composition can change drastically with a shift from CT-TPR to some form of alternative tillage and rice establishment methods (Singh *et al.*, 2009b). Tomita *et al.* (2003) observed more species-rich vegetation and diverse weed flora in Dry-DSR than in CT-TPR. A total of 46 species were present in transplanted rice in 1989, and, after 3 years (six seasons of rice) of Wet-DSR, 21 new weed species were added to the weed flora (Azmi and Mashor, 1995; Mortimer and Hill, 1999). Kim *et al.* (1992) observed a diversity index of 0.118 in Korean CT-TPR compared with 0.317 in Dry-DSR. In a study conducted in Modipuram, India, Singh *et al.* (2009b) reported that the number of species of grasses, broadleaves, and sedges was 6, 4, and 4, respectively, in CT-TPR, whereas, in Dry-DSR, it increased to 15 grass species, 19 broadleaf species, and the number of sedge species remained unaffected. This clearly shows that some new grass and broadleaf species that were not adapted to CT-TPR appeared in Dry-DSR. Higher numbers and more diverse flora in Dry-DSR could result in lower efficacy of weed management strategies, including herbicides.

In addition, adopting DSR may result in weed flora shifts toward more difficult-to-control and competitive grasses and sedges. For example, in Malaysia, at the time of the introduction of direct seeding (Wet-DSR) in the 1970s, easy-to-control broadleaf weeds were dominant but, by the 1990s, grass species such as *Echinochloa crus-galli*, *Leptochloa chinensis* (L.) Nees, and *Ischaemum rugosum* Salisb. became dominant (Azmi *et al.*, 1993, Azmi *et al.*, 2005). Similar shifts in weed flora were reported by Ho and Itoh (1991) in Malaysia when rice crops shifted from CT-TPR to Dry- and Wet-DSR. In a long-term and more detailed field study conducted in Malaysia, weedy rice and *L. chinensis* were absent in Wet-DSR plots at the start of the experiment in 1989. However, *L. chinensis* appeared after only 2 years (in 1991) and weedy rice after 4 years (in 1993) of experimentation. By 2001, weedy rice, *Echinochloa* spp., *L. chinensis*, and *Fimbristylis miliacea* became the dominant species (Azmi and Mashor, 1995; Mortimer and Hill, 1999). In Vietnam also, shifts toward more difficult-to-control grass weed species (*E. crus-galli, L. chinensis*, and weedy rice) were observed with the introduction of DSR (Chin *et al.*, 2000). Vongsaroj (1997) reported dicotyledonous weeds as dominant in transplanted rice, but annual grasses such as *E. crus-galli* and *L. chinensis* and sedges such as *F. miliacea* in DSR fields in Vietnam. Similar shifts have also been reported in India. Singh *et al.* (2005) observed that *E. crus-galli, Commelina diffusa* Burm. f.*, Cyperus rotundus* L., *Cyperus iria*, and *L. chinensis* were dominant in non-weeded Dry-DSR plots in comparison with *C. iria*, *Echinochloa colona*, and *Caesulia*

axillaris Roxb. in CT-TPR plots after four seasons of rice cropping. Direct seeding also favors sedges such as *Cyperus difformis*, *C. iria*, *C. rotundus*, and *F. miliacea* (Azmi and Mashor, 1995; Gressel, 2002; Mortimer and Hill, 1999; Yaduraju and Mishra, 2005).

Therefore, it is important that a systematic weed monitoring program be put in place along with the introduction of DSR. This would make it possible to develop adequate IWM strategies, including identification of new herbicides that are effective against a wide spectrum of weeds.

7.3. Evolution of herbicide resistance

In countries where DSR is widely adopted, herbicide use increased steadily, resulting in the appearance of resistance in weeds against certain herbicides (Table 14). For example, in Malaysia, the first case of herbicide resistance was reported in *F. miliacea* against 2,4-D in 1989. But, currently, the numbers of weed biotypes resistant to different herbicides have increased to 10. Similarly, in Thailand, Korea, and the Philippines, the number of herbicide-resistance cases in weeds increased from none before DSR introduction to 5, 10, and 3, respectively, after DSR introduction. Although no herbicide resistance case has yet been reported in South Asia, preventive measures should already be considered.

8. Breeding Cultivars for Direct-Seeded Rice

Almost no varietal selection and breeding efforts have been made for developing rice cultivars suitable for alternate tillage and establishment methods, especially in unpuddled or zero-tillage soil conditions with direct seeding (Dry-DSR) in Asia (Fukai, 2002; Lafitte *et al*., 2002). Currently, no varieties are available that are targeted for this environment though there have been successful breeding programs for direct-seeded rice in puddled conditions (Wet-DSR) (Wah, 1998; Weerakoon *et al*., 2011). On-station and on-farm trials involving the evaluation of Dry-DSR cultivation have used rice varieties/hybrids that are primarily bred for puddled transplanting. Therefore, one can argue that comparisons of crop performance of rice in Dry-DSR with CT-TPR varieties have been biased. Based on the constraint analysis discussed in the preceding section, a plant type for Dry-DSR should be different from one for CT-TPR. An ideal plant type should have traits to deal with problems associated with early CE, weed competition, spikelet sterility, and lodging. We discuss these traits in detail with an aim to identify a suitable plant type for unpuddled or zero-tillage soil conditions with direct seeding (Dry-DSR).

Table 14 Evolution of herbicide resistance in Asian countries where direct-seeded rice is predominant

Country	Year of first resistance case reported	Resistance cases before DSR introduction (no.)	Total resistant biotypes (no.)	Total resistant weed species (no.)	Resistant weed species and year of appearance
Korea	1998	0	10	8	*Monochoria korsakowii* (1998), *M. vaginalis* (1999), *Lindernia dubia* (2000), *Scirpus juncoides* var. *ohwianus* (2001), *C. difformis* (2002), *Sagittaria pygmaea* (2004), *E. oryzicola* (2002), *Scripus maritimus* (2006)
Malaysia	1989	0	10	7	*Fimbristylis miliacea* (1989), *Ischaemum rugosum* (1989), *Sphenoclea zeylanica* (1995), *Limnocharis flava* (1998), *Sagittaria guyanensis* (2000), *Bacopa rotundifolia* (2000, 2001), *Limnophila erecta* (2002)
Philippines	1983	0	3	2	*Sphenoclea zeylanica* (1983), *E. crus-galli* (2005)
Sri Lanka	1997	0	2	2	*E. crus-galli* (1997), *Ischaemum rugosum* (2004)
Thailand	1998	0	5	3	*E. crus-galli* (1998), *Sphenoclea zeylanica* (2000), *L. chinensis* (2002)

Sources: Compiled from Heap (2010) and Sangakkara *et al*. (2004).

8.1. Anaerobic germination and tolerance of early submergence

Because rice is mainly grown during the monsoon season, establishment of direct-seeded rice can be adversely affected by untimely extended rains immediately after sowing. Emergence is poor if continuous rain prevails immediately after sowing or because of the mortality of young seedlings caused by submergence (Ismail *et al.*, 2009). Therefore, ability to germinate under anaerobic conditions and tolerance of early submergence are important for establishing a good crop (Ismail *et al.*, 2009). This trait may help in weed suppression too in areas where water is available by allowing early flooding. Although it is known that submergence tolerance is often associated with lower yield, McKenzie *et al.* (1994) were able to combine both submergence tolerance and high yield.

8.2. Early vigor

Good seed quality and seedling vigor are desirable for optimal establishment of a DSR crop, and also for weed competitiveness (Redoña and Mackill, 1996). Seedling vigor is defined as the ability of a plant's aerial part to emerge rapidly from soil or water (Heydecker, 1960). Rapid germination, rapid shoot and root growth, and long mesocotyls and coleoptiles are important seedling vigor-related traits (Cui *et al.*, 2002; Redoña and Mackill, 1996; Sasahara *et al.* 1986; Williams and Peterson, 1973). All these traits will favor seedling establishment in direct seeding. For example, rapid germination and rapid shoot development are likely to help in avoiding submergence stress. A longer mesocotyl will minimize sensitiveness to seeding depth in drill seeding and improve seedling establishment. The modern semi-dwarf cultivars have a short mesocotyl, and this is disadvantageous for good CE, especially when seeds are drilled deeper in the soil (Dilday *et al.*, 1990; Fukai, 2002; Turner *et al.*, 1982). In the absence of precise land leveling and precise seeding machinery, it is difficult to achieve precise placement of seeds at shallow depth. Therefore, a suboptimal sowing depth leads to poor CE. Moreover, in conservation tillage systems in which residue is mulched, emergence of the crop may be adversely affected because of short mesocotyl.

8.3. Crop competitiveness against weeds

This is one of the most important plant traits required for the success of DSR. As discussed earlier, weeds are a major constraint in DSR cultivation. The development of weed-competitive cultivars is an attractive low-cost strategy of an overall IWM program for both low- and high-input cropping

systems (Cousens, 1996; Dingkuhn *et al.*, 1999), and the most efficient way of delivery to farmers (Caton *et al.*, 2003).

Cultivar differences in weed competitiveness have been reported in many crops, including rice (Chavez, 1989; Fischer *et al.*, 2001; Garrity *et al.*, 1992; Haefele *et al.*, 2004; Quintero, 1986). Cultivar–weed competitiveness has two components: weed tolerance and weed-suppressive ability (Jannink *et al.*, 2000; Zhao *et al.*, 2006). Weed tolerance is the ability of plants to maintain high yields despite weed competition, whereas weed-suppressive ability is the ability to suppress the growth of weeds through competition. Breeding for weed-suppressive ability is being advocated over weed tolerance because suppressing weed growth will reduce weed seed production and minimize contributions to the weed seed bank (Jannink *et al.*, 2000; Jordan, 1993).

Rice characteristics reported to be associated with weed competitiveness include (a) plant height together with early and rapid growth rate (Caton *et al.*, 2003; Garrity *et al.*, 1992), (b) higher tiller number (Fischer *et al.*, 1997), (c) droopy leaves (Dingkuhn *et al.*, 1999), (d) relatively high biomass accumulation at the early stage (Ni *et al.*, 2000), (e) high leaf area index (Dingkuhn *et al.*, 1999) and high specific leaf area (Audebert *et al.*, 1999; Dingkuhn *et al.*, 1999) during vegetative growth, (f) rapid canopy ground cover (Lotz *et al.*, 1995), and (g) early vigor (Zhao *et al.*, 2006). It is argued that the introduction of some of these traits in a variety may result in some yield loss (Dingkuhn *et al.*, 1999; Jennings and Jesus, 1968; Kawano *et al.*, 1974; Pérez de Vida *et al.*, 2006). However, it is also argued that the benefit of having these traits is likely to be higher than when not having them (Fischer *et al.*, 2001; Garrity *et al.*, 1992; Gibson *et al.*, 2003; Ni *et al.*, 2000; Zhao *et al.*, 2006).

Although tall plants are linked to weed competitiveness, they often have low yield potential and tend to lodge. Fischer *et al.* (1997) also reported that semi-dwarf varieties can be as competitive as tall plant-type varieties. Therefore, shorter intermediate height (between tall traditional and modern semi-dwarf) may be more desirable for direct seeding (Fukai, 2002). Unlike an initial shock in transplanting that delays tillering, tillering does not seem to be a constraint in direct seeding. Therefore, tillering ability is not a primary trait for selection (Dingkuhn *et al.*, 1990; Fukai, 2002; Song *et al.*, 2009). In fact, Song *et al.* (2009) reported that excessive tillering at an early stage could result in reduced leaf biomass and photosynthesis at a later stage and eventually become one of the major reasons for lower yields. *Oryza glaberrima*, a cultivated rice with low yield potential, possessing the trait of droopy leaves with high specific leaf area, is very effective in weed suppression. Jones *et al.* (1997a,b) suggested that, if this trait is restricted to early growth and combined with the trait of erect leaves with low specific leaf area from *O. sativa*, this can be useful for direct seeding.

Dingkuhn *et al.* (1999) suggested that the trade-off between weed competitiveness and high yield potential can be reduced by expressing weed competitiveness traits at an early development stage only. Through path analysis, Pérez de Vida *et al.* (2006) found that (a) early growth and light-capturing traits followed by moderate growth rates before heading and (b) a vigorous grain-filling period (high rate of grain-filling and long grain-filling duration) are ideal for both weed competitiveness and high yield potential. Therefore, an ideal plant type with an ability to compete against weeds would have early seedling vigor with shoots to quickly spread and cover the ground during the vegetative stage. It is thought that these characteristics theoretically result in a high radiation extinction coefficient, leading to high light use efficiency, and eventually result in the suppression of weeds. Further, Jones *et al.* (1997a,b) and Dingkuhn *et al.* (1999) argued that cultivars having high specific leaf area during vegetative growth and low specific leaf area with high chlorophyll content during the reproductive phase are compatible with high yield and weed competitiveness.

8.4. High crop growth rate during the reproductive phase

A slower crop growth rate during the reproductive phase has been reported to be associated with poor spikelet fertility, which is a most commonly observed characteristic in direct seeding. Horie (2001) reported that crop growth rate during the 2-week period preceding full heading determines yield through effects on spikelet number, single-grain mass, and potential grain-filling. The rice plant enters the reproductive phase about 1 month before anthesis and generally differentiates excess spikelets depending on previous N uptake. Spikelets then degenerate during this 2-week period preceding full heading depending on the availability of carbohydrates (Matsushima, 1957; Wada, 1969). Kato and Katsura (2010) observed that the frequency of floret abortion was associated with biomass production during the reproductive phase. This suggests that, in order to achieve high panicle fertility, sink demand should be met by high canopy photosynthesis at pre-anthesis and high remobilization ability.

Causes of low crop growth rate during the reproductive phase in direct seeding may be attributed to (a) high biomass during the vegetative phase and thus more maintenance respiration, (b) low foliar N concentration, and (c) reduced canopy CO_2 assimilation rates (Dingkuhn *et al.*, 1992; Yoshida, 1981). The low growth rate during the reproductive phase of direct-seeded rice leads to its earlier senescence than transplanted rice (Dingkuhn *et al.*, 1991a,b**)**. Thus, a plant type with erect leaves having low specific leaf area (higher biomass per unit area) and high chlorophyll content (Dingkuhn *et al.*, 1999), which is likely to increase the crop growth rate during the reproductive phase (Horie *et al.*, 2004) and prolong the ripening phase

(Dingkuhn *et al.*, 1991a,b), has desirable characteristics for direct seeding. Katsura *et al.* (2010) found higher yield in direct-seeded aerobic rice than in puddled transplanted rice because of high N accumulation during the ripening phase. Thus, the ability to enhance N uptake during the ripening phase, which is a prerequisite to enhancing canopy photosynthesis and assimilate supply, is equally important to be considered in a breeding program for direct seeding (San-oh *et al.*, 2004). In addition, direct-seeded rice cultivars must possess enhanced assimilate export ability from the vegetative parts to reproductive parts during the reproductive phase (Dingkuhn *et al.*, 1991a,b).

8.5. Modified panicle architecture

Direct seeding, especially under dry conditions, can have the risk of dry spell even under irrigated conditions, which could adversely affect spikelet formation and development. Grain number per unit area, an important variable, is highly influenced by genotype and environment interaction. More grains in the primary branch of the panicle together with apical-borne spikelets are a stable trait and not much influenced by environment. Hence, selecting a genotype with more primary branches per panicle with more contribution by the primary branch apical-borne spikelets can provide some buffering to overcome the adverse effects of a dry spell during the reproductive period, and low-input level of water and nutrients (C.K. Reddy personal communication).

8.6. Modified root system

There are visible differences in rooting pattern of direct-seeded or transplanted rice plants. Kato and Okami (2010) found lower root biomass in Dry-DSR than in CT-TPR owing to a reduction in root biomass in the surface soil (fewer adventitious roots). However, the ratio of deep root to total root biomass was higher in Dry-DSR. Vigorous growth of superficial roots has been linked with the better performance of high-yielding lowland-adapted cultivars (Morita and Yamazaki, 1993). Therefore, in addition to deeper roots, vigorous adventitious surface rooting would be beneficial to improving N and water uptake efficiencies, especially during reproductive growth. Roots also play an important role in cytokinin synthesis. Cytokinin synthesis is enhanced in plants with a well-developed root system or when the physiological activity of roots is high (Soejima *et al.*, 1992, 1995). The rate of leaf senescence is low in plants in which a large amount of cytokinin is transported from the roots to the shoot (Soejima, 1992, 1995).

8.7. Lodging resistance

Lodging under DSR can be relatively more problematic than in CT-TPR because of the higher seed rate in DSR and when DSR is broadcast/surface-seeded. Therefore, lodging resistance is another desirable trait for direct seeding. Intermediate plant height, large stem diameter, thick stem walls, and high lignin content are traits of lodging tolerance (Mackill *et al.*, 1996). In addition, lower positioning of panicles in the plant's canopy is known to be associated with increased tolerance of lodging (IRRI, 1994; Setter *et al.*, 1997).

8.8. Shorter-duration rice cultivars

Rice grain yields have been reported similar or higher with direct seeding than with transplanting when shorter-duration rice cultivars were used (Dingkuhn *et al.*, 1991a,b). Shorter duration of the crop also allows integration of more and different crops to enhance intensification/diversification of the production system.

9. A Dry Direct Drill-Seeded Rice Technology Package for the Major Rice-Based Systems in South Asia

Dry direct drill seeding has great potential in South Asia as an alternative to the conventional practice of puddled transplanting to overcome emerging resource constraints, especially labor, water, and energy shortages, and to address the increasing cost of cultivation. However, the performance of Dry-DSR has not yet reached its full potential in South Asia, primarily because of the unavailability of a complete Dry-DSR production technology package. Both rice genotype development and resource management are critical for achieving optimal production under Dry-DSR. Earlier, we discussed potential plant traits whose selection could lead to an efficient plant type for DSR. In this section, we review the large quantity of work on resource management that has been carried out during the past decades by the Rice-Wheat Consortium of the Indo-Gangetic Plains (RWC) (Gupta *et al.*, 2006; Ladha *et al.*, 2009; RWC-IRRI, 2009) and elsewhere (Bazaya *et al.*, 2009, Prasad, 2005; Sen and Sharma, 2002). At least two major publications are available that describe a technological package for Dry-DSR (Gopal *et al.*, 2010; Gupta *et al.*, 2006). Here, we provide the current status of work and salient recommendations for growing a successful crop of Dry-DSR.

The most important prerequisites for a successful crop of dry direct drill-seeded rice are (1) precise land leveling, (2) good CE, (3) precise water management, and (4) effective and efficient weed management. These are discussed in detail below.

9.1. Precise land leveling

Good land leveling is an entry point for DSR because it (1) facilitates uniform and good CE, (2) permits precise and uniform water control and good drainage, (3) reduces the amount of irrigation water needed, (4) increases cultivation area because of fewer bunds, (5), improves input use efficiency (water, nutrients, and agrochemicals), and (6) increases crop productivity (Jat *et al.*, 2006, 2009; Lantican *et al.*, 1999; Rickman, 2002). In the Philippines, Lantican *et al.* (1999) observed correlation between DSR yield and precision of land leveling. They estimated an average yield loss of 0.9 t ha^{-1} due to deficiency in land leveling, which results primarily from water stress in areas not leveled.

Studies conducted by the RWC reported a widespread problem of poor leveling in South Asia (Jat *et al.*, 2006). Fields leveled by traditional methods generally have large variability across the field with frequent dikes and ditches. The average field slope in the IGP varies from 1° to 3° in the northwest (India and Pakistan) and from 3° to 5° in the eastern region (eastern India, Nepal, and Bangladesh). Due to a lack of uniform water distribution associated with unevenness of land, the problem of excess or no water causing large yield variability within a field is common. In 2001, laser-assisted precision land leveling was introduced as an entry point for the success of alternative tillage and CE practices in the region. It allowed planters/drills to place seed at a uniform distance and depth, and enabled uniform distribution of irrigation water across the field, resulting in uniform crop stand. Leveling of 1.5–2 cm of standard deviation has been recommended for dry drill seeding after zero-tillage (Kawasaki, 1989; Kimura *et al.*, 1999). Laser land leveling is the single most popular technology in the IGP, where it has spread rapidly on about 1.0 million ha in India (M. L. Jat personal communication) and 0.16 million ha in Pakistan (Ladha *et al.*, 2009).

9.2. Crop establishment

Uniform crop emergence with optimum plant density is crucial for achieving good yields for any system, including direct drill-seeded rice. Good CE depends on many factors, including land preparation, planting date, seed rate and seed preparation, types of planting machinery used, and depth of seeding.

9.2.1. Land/seedbed preparation

The method of seedbed preparation differs for conservation (reduced or zero-till) and conventional-till systems. But, for both, the seedbed should be free of weeds and precisely leveled at the time of sowing. For conventional-till dry drill seeding (CT-dry-DSR), the soil should be well pulverized to maintain good soil moisture for drilling and good soil-to-seed contact. In sandy or silt loam, an excellent seedbed can be prepared with reduced or minimum tillage, thereby conserving soil, and reducing cost. In zero-till dry drill seeding (ZT-dry-DSR), it is important to first knock down the existing vegetation (annual and perennial weeds) with a burndown herbicide such as paraquat (0.5 kg ai ha^{-1}) or glyphosate (1.0 kg ai ha^{-1}).

9.2.2. Planting dates

Rice in South Asia is mainly grown during the monsoon season (wet season). In India and Nepal, it is commonly known as *kharif* and in Bangladesh as the *aman* season. To effectively use monsoon rain, the optimum time for planting wet-season rice is about 10–15 days prior to the onset of monsoon (based on forecast or historical weather data) (Gopal *et al*., 2010; Gupta *et al*., 2006). After the onset of rain when soil gets wet, movement of machinery becomes difficult, which makes seeding tedious. Moreover, if rain continues for a few days, seed rotting and seedling mortality can occur due to submergence, resulting in poor CE. Based on the historical trend of the onset of monsoon in different areas in the region, the optimum time for seeding rice is given in Table 15.

Table 15 Optimum sowing time in relation to onset of monsoon for dry direct-seeded rice in South Asia

Area	Onset of monsoon	Optimum time of seeding
Punjab, India	July 1–15	Mid-June to third week of June
Haryana, India	June 20–July 1	First fortnight of June
Western Uttar Pradesh, India	June 20–July 1	First fortnight of June
Eastern UP and Bihar, India	June 10–15	Last week of May to early June
West Bengal, India	June 1–15	Last week of May
Tarai, Nepal	June 15–July 7	End of May to mid-June

Source: Modified from Gopal *et al*. (2010).

9.2.3. Priming of seeds and seed treatment

Priming of seeds has been shown to have positive effects on the emergence, yield, and quality of dry direct-seeded rice (Farooq *et al.*, 2006a,b; Harris *et al.*, 2002). In dry drill seeding, good CE is constrained by subsurface soil drying associated with high temperature. Hence, priming of seeds (prehydration) offers the advantage of early and improved emergence, and early vigor. Priming is accomplished by soaking seeds in water for 10–12 h and then drying them in shade prior to seeding. This process facilitates a free flow of seed during seeding operations. However, seeds should be sown shortly after priming to avoid deterioration. Emergence of primed seeds will be affected if seeds encounter moisture stress initially. Therefore, seeding with primed seeds should be done only after pre-sowing irrigation.

Seed rot and seedling mortality are caused by various soil- and seed-borne fungi or other pathogens such as termites and nematodes (Krausz and Groth, 2008). Fungicide and/or insecticide seed treatments have been shown to improve the crop stand in many crops, including in dry drill seeding of rice (Krausz and Groth, 2008). Insecticides such as imidacloprid (Gaucho 70 WS) and thiamethoxam (Cruiser 5 FS) and fungicides such as carbendazim, streptocycline, metalaxyl, thiram, and mancozeb can be used for seed treatment (Gopal *et al.*, 2010; Gupta *et al.*, 2006; Tarun Sharma, personal communication). Both dry and primed seed can be treated. For primed seed, treatment with fungicide or insecticide should be done post-soaking. Damage by rice Thrips on emerging seedlings could also be controlled by using seed treatments.

9.2.4. Seed rate

The published literature shows a widespread use of seed rates of up to 200 kg ha^{-1} to grow a DSR crop (Guyer and Quadranti, 1985). High seed rates are used mostly in areas where seed is broadcast with an aim to suppress weeds or when water-seeded (Moody, 1977). However, it is not clearly known whether a high seed rate is primarily used to control weeds or is really a requirement to raise a good crop of DSR. Studies have reported an increase in yield only in weedy plots and not in weed-free or weeded plots with increases in seed rate. Therefore, higher seedling rates can be beneficial only in conditions with no or partial weed control (Castin and Moody, 1989; Guyer and Quadranti, 1985). Farmers also use high seed rate when conditions for germination are poor due to damage by birds, insects, rats, etc. or the germination percent of seed itself is low. The benefits of a higher panicle number associated with a higher seed rate are offset by a reduction in panicle length and grain weight per panicle (Bhattacharjee, 1978).

When using a drill for seeding, the seed rate can be decreased drastically without causing any adverse effect on yield if weeds are controlled effectively. Based on recent experience with on-farm farmer participatory trials in the IGP, a seed rate of 20–25 kg ha^{-1} has been found optimum for

medium-fine-grain rice cultivars with a spacing of 20 cm between rows and 5 cm within rows (Gopal *et al.*, 2010; Gupta *et al.*, 2006). Very few on-station studies have been conducted evaluating the effects of seed rate to assess the performance of Dry-DSR. Sudhir-Yadav *et al.* (2007) evaluated seed rates of 30, 40, and 50 kg ha^{-1} for basmati rice in Punjab, India, and found that a seed rate of 30 kg ha^{-1} yielded the highest. Wu *et al.* (2008) in China found a seed rate of 20–25 kg ha^{-1} as optimum for DSR, including under zero-till conditions (ZT-dry-DSR). However, others found no difference in yield with a range of seed rates (Gravois and Helms, 1992; Johnson *et al.*, 2003; Jones and Snyder, 1987; Xie *et al.*, 2008).

High seed rates can result in large yield losses due to excessive vegetative growth before anthesis followed by a reduced rate of dry matter accumulation after anthesis (Wells and Faw, 1978) and lower foliage N concentration at heading (Dingkuhn *et al.*, 1990). These factors result in higher spikelet sterility and fewer grains per panicle (Baloch *et al.*, 2007; Huan *et al.*, 1999; Kabir *et al.*, 2008; Tuong *et al.*, 2000). Moreover, dense plant populations at high seed rates can create favorable conditions for diseases (e.g., sheath blight; Mithrasena and Adikari, 1986; Guzman Garcia and Nieto Illidge, 1992) and insects (e.g., brown planthoppers) and make plants more prone to lodging (Dofing and Knight, 1994; Islam *et al.*, 2008). A high seed rate also increases establishment costs.

Plant spacing has a major effect on crop yields. Huan *et al.* (1999) showed that, as the seed rate increases, tillering decreases and panicle density is more dependent on primary than on secondary or tertiary tillers. Since panicles from primary tillers are more productive than those from secondary and tertiary tillers, we should target an optimal spacing to have more panicles from primary tillers by minimizing interplant competition. Much research on plant spacing is done for optimizing transplanting (De Datta, 1981). If we follow this lead, then plant spacing in direct seeding should be similar to that in transplanting, if weed control is good. This means that a high seed rate is not needed in DSR to achieve high yields. More research is needed, however, to study the interaction of seed rate, variety, seed depth, spacing, and geometry.

9.2.5. Planting machinery (drills/planters)

For accurate and precise seeding, the crop should be drilled using a multi-crop planter with a precise seed-metering system (e.g., inclined plate, cupping system, or vertical plates) (Fig. 11B–D; Gopal *et al.*, 2010; Gupta *et al.*, 2006). With these precise seed-metering planters, rice can be established with a lower seed rate and more precise plant-to-plant spacing can be maintained. Normal fluted roller-type seed-cum-fertilizer drills are less suitable for drill seeding of rice as the seeds fall continuously. This makes it difficult to maintain the seed rate and plant-to-plant spacing as accurate and precise as that in inclined-/cupping-/vertical-plate seed-metering systems (Gopal *et al.*, 2010; Gupta *et al.*, 2006). It is difficult to drill rice at a low seed rate of 20–25

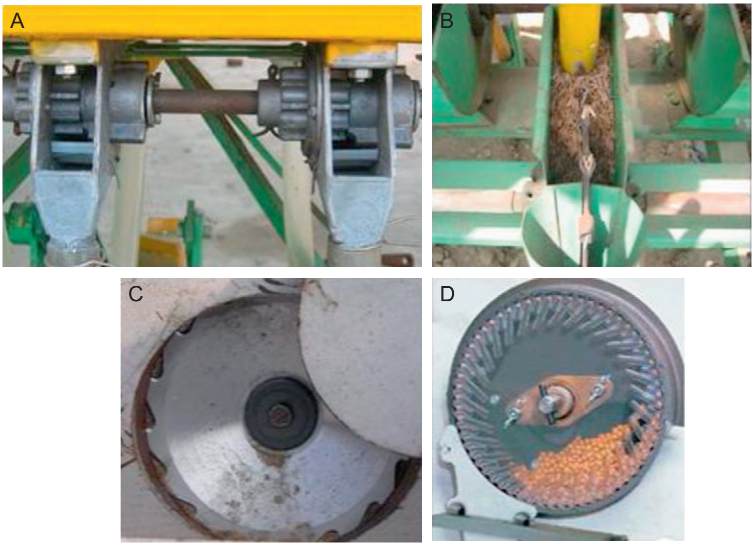


Figure 11 Multicrop planter's seed-metering systems, (A) fluted roller, (B) cup type, (C) inclined plate, and (D) vertical plate. Source: Gupta *et al.* (2006) and Gopal *et al.* (2010).

kg ha^{-1} with a fluted roller seed drill because it breaks the seeds. If farmers do not have inclined-plate planters, they can seed at a lower rate with a normal drill by mixing with sand to increase the seed volume and opening of the fluted roller so that breakage of rice can be avoided (Gopal *et al.*, 2010). *Sesbania* (a leguminous green manure) seeds can also be mixed to achieve the same purpose. *Sesbania* is then killed with 2,4-D about 30 days after seeding (Singh *et al.*, 2007). The PTOS is most commonly used for seeding rice in Nepal and Bangladesh. The PTOS has a seeding device attached to the power tiller (Chinese hand tractor). It tills the soil shallow (4–5 cm), sows seed in rows, and covers it with soil at the same time in a single pass.

Specialized machines are required for ZT-dry-DSR with loose crop residue. Recently, different machines have been evaluated and refined to seed under loose residue, especially after combine harvest in South Asia (Gopal *et al.*, 2010; Sharma *et al.*, 2008; Sidhu *et al.*, 2008; Singh, 2008). Some of the machines that can be used for seeding rice with surface residues are briefly discussed here:

9.2.5.1. Turbo seeder With this machine, rice can be drilled into a loose residue mulch load of up to 8–10 t ha^{-1} (Gopal *et al.*, 2010; M. K. Gathala, personal communication). It shreds the residues in the narrow strip in front of the tine openers and places seeds and fertilizer using an inverted-T-type opener. The seed-metering system on currently manufactured machines

comprises fluted rollers, as this machine was originally designed for sowing wheat into rice residues. Minor redesign is in progress to provide a better seeding mechanism (inclined plate) for rice.

9.2.5.2. PCR planter

This is an advanced version of the turbo seeder/planter. It has a multicrop precise seed-metering system (vertical plates) with adjustable row arrangements. It is capable of seeding into a residue load of up to 8–10 t ha^{-1} (Gopal *et al.*, 2010).

9.2.5.3. Double-disc coulters

In this machine, double-disc coulters are fitted in place of tines to place seed and fertilizer into the loose residues. This machine can drill seeds into a loose residue load of up to 3–4 t ha^{-1}. A limitation with this machine is that, being lightweight (0.3 t), it fails to cut through the residues, resulting in some seed and fertilizer being placed on the surface of residues (Gopal *et al.*, 2010; Sharma *et al.*, 2008).

9.2.5.4. Rotary-disc drill

It is based on a rotary-till mechanism. It is mounted on a three-point linkage system and is powered through the power take-off shaft of the tractor. The rotor is a horizontal transverse shaft having six to nine flanges fitted with a straight disc for a cutting effect while rotating at 220 rpm (Singh *et al.*, 2008). The rotating discs cut the residue and make a narrow slit into the soil to facilitate placement of seed and fertilizer. This machine can be used under loose residue, anchored residue, and residue-free conditions. It can handle a residue load of 7–8 t ha^{-1}. This machine's limitation is the blunting of front-powered discs, which, however, can be overcome by using discs of greater strength (Sharma *et al.*, 2008).

9.2.6. Depth of seeding and moisture

Seeding depth is critical for all rice varieties but more so for semi-dwarf plant types because of their shorter mesocotyl length compared with conventional tall varieties (Blanche *et al.*, 2009). Therefore, rice should not be drilled deeper than 2.5 cm to maximize uniform CE. It is important to have sufficient moisture during the germination period. As sowing is done during peak summer when the open-pan evaporation rate is as high as 8–12 mm day^{-1}, the soil surface can dry very quickly and the seed zone can experience moisture stress (Gopal *et al.* 2010; Tabbal *et al.*, 2002).

Rice can either be drilled in dry soil followed by a light irrigation or drilled in moist soil after preirrigation to ensure good emergence and uniform establishment. In the latter situation, planking after seeding will conserve soil moisture and improve soil-to-seed contact.

9.3. Precise water management

Precise water management, particularly during CE phase (first 7–15 days after sowing), is crucial in dry drill-seeded rice (Balasubramanian and Hill, 2002; Kumar *et al.*, 2009). From sowing to emergence, the soil should be kept moist but not saturated to avoid seed rotting. After sowing in dry soil, applying a flush irrigation to wet the soil if it is unlikely to rain followed by saturating the field at the three-leaf stage is essential (Bouman *et al.*, 2007). This practice will not only ensure good rooting and seedling establishment but also enhance the germination of weed seeds. Therefore, early weed control with an effective preemergence herbicide is very important to check weed emergence and growth.

As already discussed, precise leveling is crucial for the uniform spread of water as well as easy drainage which is needed during the CE phase of Dry-DSR. When water control and/or drainage are poor, the crop is likely to fail due to submergence in the early stage. Bund management also plays an important role in maintaining uniform water depth and limiting water losses via seepage and leakage (Lantican *et al.*, 1999; Tuong *et al.*, 1994). It is important that the bunds be prepared as soon as possible after sowing, which includes compacting and plastering of any holes or cracks.

Information on irrigation and water management in Dry-DSR is scarce (Humphreys *et al.*, 2010). Gupta *et al.* (2006) and Gopal *et al.* (2010) recommended avoiding water stress and keeping the soil wet at the following stages: tillering, panicle initiation, and grain filling. Bouman *et al.* (2007) suggested keeping the field flooded 1 week before and after peak flowering to avoid water stress around flowering, the most sensitive stage of rice to water stress. After CE, the following four broad water management options are available: (1) continuous flooding; (2) frequent irrigation, that is, DSR with safe alternate wetting and drying (AWD), which involves flooding the field with shallow depth (5 cm) and reirrigating a few days after water disappearance; (3) infrequent irrigation where scarcity of irrigation water limits rice yields; and (4) no irrigation under rainfed conditions (Humphreys *et al.*, 2010). Given the aim of achieving high yields of Dry-DSR with less water, option 2 is preferred but this is subject to the availability of irrigation water. Like CT-TPR, Dry-DSR can also be irrigated using safe AWD to economize in water use. However, our knowledge in terms of optimal soil water status to implement safe AWD in Dry-DSR is still limiting. Nevertheless, farmers and researchers provide many anecdotal reports indicating that a safe AWD irrigation interval in Dry-DSR is longer than that in CT-TPR because of less soil cracking in the former than in the latter (Humphreys *et al.*, 2010). In a 6-year study conducted in Modipuram, India on sandy-loam soil, it was observed that Dry-DSR can be irrigated safely at the appearance of soil hairline cracks (Bhushan *et al.*, 2007; Gathala *et al.*, 2011). This study

recorded an average savings of 9% irrigation water when irrigation took place on the appearance of soil hairline cracks (this coincided with −25 to −35 kPa at 15-cm depth). Another study conducted by Sudhir-Yadav *et al.* (2011a,b) in Punjab, India on clay loam soil observed −20 kPa soil tension at 20 cm depth as safe for AWD irrigation scheduling. They observed 33–53% irrigation water saving in Dry-DSR with AWD compared with CT-TPR without compromising grain yield. Further research is needed to determine the optimum threshold for irrigation at different growth stages and for a wider range of rainfall and evaporative demand conditions and varietal types. Moreover, Dry-DSR with residue mulch would also require appropriate irrigation scheduling and water management as residue mulch would influence evaporation, infiltration, and transpiration very differently than conventional practice.

A large area of the rice–wheat cropping system of South Asia is irrigated primarily from groundwater. Any attempt to reduce deep drainage losses in these areas would neither save water nor reduce groundwater decline (Humphreys *et al.*, 2010) because often that water is reused/pumped. However, reductions in deep percolation losses can save energy (energy needed to pump) and reduce groundwater pollution. To have a significant impact on true water savings, we need technologies that can reduce ET and increase water productivity of evapotranspired water (WP_{ET}) (Humphreys *et al.*, 2010). For example, residue mulch in Dry-DSR may significantly reduce *E* and ET, especially prior to the start of monsoon when evaporation is very high and plants are very small (Jalota and Arora, 2002). The development of new cultivars of short to medium duration adapted to water limitations is another approach to reducing irrigation water use (Humphreys *et al.*, 2010).

Recently, interest has been increasing in using pressurized irrigation method to grow rice in areas where water is becoming scarce (Spanu *et al.*, 1996). Limited studies in the region have shown that sprinkler systems have potential to improve on-farm irrigation efficiency up to 80% in other crops under the prevailing conditions in the Indian subcontinent (Sharma, 1984). Sprinkler systems can be used in rice to apply a desired depth of water during pre- and post-sowing irrigations (Kahlown *et al.*, 2007). In Pakistan, Kahlown *et al.* (2007) found that sprinkler irrigation increased the grain yield of CT-TPR by 18% and reduced water application by 35% compared with the traditional irrigation system. Similarly, Kato *et al.* (2009) in Japan found that Dry-DSR when irrigated with a sprinkler system (30–40 mm) whenever soil water potential fell below −60 kPa at 20-cm depth produced equal or higher yield than transplanted or dry direct-seeded rice under a flooded system, with total water savings ranging from 21% to 74%. Although some of these studies show potential, much needs to be done to understand the feasibility and economics of pressurized irrigation methods in farmers' fields when land holdings are small. This area seems to have huge

untapped potential which should be explored in close collaboration with various partners, especially in the private sector.

9.4. Effective and efficient weed management

IWM is desirable for effective and sustainable weed control in Dry-DSR (Rao and Nagamani, 2007; Rao *et al.*, 2007). Effective IWM integrates many "little hammers" instead of a single "large hammer" (e.g., herbicides) to control a wide range of weeds at many points in their life cycle (Liebman and Gallandt, 1997). Tools available for IWM can be categorized broadly into (a) cultural, (b) chemical, (c) mechanical, and (d) biological controls. Here, we review the published studies that have shown effective management strategies that can be integrated to manage weeds in Dry-DSR. IWM can also be enhanced through an understanding of the biology and ecology of specific problematic weeds to help identify weak points in weed life histories that can be efficiently targeted for management.

9.4.1. Cultural practices

9.4.1.1. The stale seedbed technique In this technique, after seedbed preparation, the field is irrigated and left unsown to allow weeds to germinate. Following emergence, weeds are killed either by a nonselective herbicide (usually paraquat or glyphosate) or by carrying out tillage prior to the sowing of rice. This technique not only reduces weed emergence but also reduces the number of weed seeds in the soil seedbank (also referred to as the soil weed seedbank) (Rao *et al.*, 2007). Singh *et al.* (2009b) reported 53% lower weed density in Dry-DSR after a stale seedbed than without this practice. The success of stale seedbeds depends on several factors: (a) method of seedbed preparation, (b) method of killing emerged weeds, (c) weed species, (d) duration of the stale seedbed (Ferrero, 2003), and (e) environmental conditions (e.g., temperature) during the stale seedbed period. Weed species, especially *C. iria*, *C. difformis*, *F. miliacea*, *L. chinensis*, and *Eclipta prostrata* (L.), can be relatively more susceptible to the stale seedbed technique combined with zero-till because of their low seed dormancy and their inability to emerge from a depth greater than 1 cm (Chauhan and Johnson, 2008a,b; Chauhan and Johnson, 2009, 2010). Renu *et al.* (2000) found that a stale seedbed with herbicide (paraquat) was more effective in weed suppression than with the mechanical method in Dry-DSR because herbicides kill weeds without bringing new seeds to the germination zone. Ideally, the duration of the stale seedbed should be long enough to allow the maximum emergence of weeds to the two- to three- leaf stage. However, in practice, the duration of the stale seedbed may be determined by the optimal planting timing for rice.

9.4.1.2. Land preparation—tillage and leveling Land preparation including tillage and precise land leveling before crop planting plays an important role in controlling weeds in dry drill-seeded rice. Tillage determines the vertical distribution of weed seeds in the soil profile, which in turn affects seedling establishment depending on factors such as seed predation, seed dormancy, seed longevity, and the potential of seedlings to emerge from a given depth (Chauhan *et al*., 2006). Zero tillage can reduce weed problems and make management easier if weeds are managed effectively in the initial 2–3 years. Zero tillage may also reduce weed emergence of some species as the seeds at the soil surface are more prone to predation (Jacob Spafford *et al*., 2006) and desiccation (Mohler and Galford, 1997). In addition, the physical environment created by surface residue in a ZT system provides a habitat for weed seed predators and also offers conditions more conducive to microbial decay of weed seeds because of more microbial activity (Gallandt, 2006; Gallandt *et al*., 1999). Therefore, for annual weeds (reproduced primarily by seeds), reduced tillage may result in reduced weed seed survival and emergence in the long run with the assumption that weed seed production is not increased in reduced-tillage systems. However, for perennial weeds [reproduce vegetatively, or through underground tubers (e.g., sedges)], a lack of tillage may exacerbate weed problems if weeds are not controlled effectively by a nonselective herbicide (glyphosate) prior to crop planting. In situations where weed control is suboptimal and the weed seed load is relatively high, conventional tillage may be a more suitable option as tillage can bury weed seeds below germination zones and can reduce weed problems.

Precise land leveling helps improve weed control by enabling precise water control and improving herbicide efficiency. This has been shown to be effective in reducing the weed population up to 40%, the labor requirement for weeding by 75% (16 person-days ha^{-1}), and weeding cost by 40% (Rickman, 2002). By and large, land leveling has been overlooked as an option for managing weeds. More work in this area would clarify the exact role of land leveling in weed dynamics and composition.

9.4.1.3.* Sesbania *coculture *Sesbania* is a legume used as a green manure in rice cultivation either as pre-rice or an inter- or mixed crop with rice (Singh *et al*., 2009b). It is sown at 25 kg ha^{-1} together with rice. After 25–30 days of growth, when *Sesbania* is about 30–40 cm tall, it is killed with 2,4-D ester at 0.50 kg ha^{-1}. This coculture technology can reduce the weed population by nearly half without any adverse effect on rice yield. Singh *et al*. (2007) reported that *Sesbania* coculture reduced broadleaf and grass weed density by 76–83% and 20–33%, respectively, and total weed biomass by 37–80% compared with a sole rice crop. This may largely be due to the rapid growth of *Sesbania* and, to some extent, mulch effects of its biomass.

The effectiveness of this technique is further enhanced by the application of pendimethalin, a preemergence herbicide. Pendimethalin is effective in controlling grass weed species, which otherwise become difficult to control after knockdown of *Sesbania* because of their large size. *Sesbania* followed by 2,4-D was more effective in suppressing broadleaves and sedges and less effective on grasses. Therefore, it is recommended to use pendimethalin as a preemergence to overcome the problem of grass control in this technique.

In addition to weed suppression, other benefits of *Sesbania* coculture are atmospheric nitrogen fixation and facilitation of crop emergence in areas where soil crust formation is a problem (Gopal *et al.*, 2010; Singh *et al.*, 2009b). Despite these benefits, *Sesbania* coculture may pose risks of competition with rice if 2,4-D application is ineffective or 2,4-D application is delayed due to continuous rain and could also increase the cost of production. Moreover, *Sesbania* coculture may limit the use of herbicides as some of these herbicides may kill *Sesbania* also.

9.4.1.4. Residue mulching Retaining crop residue on the soil surface as mulch can suppress weeds by reducing the recruitment of seedlings and early growth. Residue mulch can suppress weeds by (a) providing a physical barrier to emerging weeds (Mohler, 1996; Mohler and Callaway, 1991; Mohler and Teasdale, 1993) and (b) releasing allelochemicals in the soil with decomposition (Chou, 1999; Weston, 1996). Limited research has been done in rice-growing regions to determine the potential of mulching for weed suppression in DSR. A study conducted in India found that wheat residue mulch of 4 t ha^{-1} reduced the emergence of grass weeds by 44–47% and of broadleaf weeds by 56–72% in dry drill-seeded rice (Singh *et al.*, 2007). This reduction in weed emergence resulted in 17–22% higher grain yield in mulched plots compared with unmulched plots. Information on the amount of residue required to suppress weeds without hindering CE is lacking. In a pot experiment in the Philippines, Chauhan and Johnson (2010) reported that rice residues ranging from 2 to 6 t ha^{-1} inhibited and delayed the emergence and biomass of many rice weed species, including *E. colona*, *E. crus-galli*, *Digitaria ciliaris*, *Dianella longifolia*, and *Eleusine indica*. In actual field conditions, higher amounts of crop residue may be required to be effective in weed suppression. In South Asia, there is a competition for crop residue with animal feed (RWC-CIMMYT, 2003). The retention of crop residues has also been advocated in the intensive rice–wheat system in the region because of increasing concern about depleting soil organic matter and environmental pollution with the burning of crop residues (Gupta *et al.*, 2006).

Because of puddling and the flooding nature of conventional rice culture, not much attention has been paid to the use of residue mulch, including in DSR. However, zero tillage followed by direct seeding provides an opportunity to use residue as mulch. In combine-harvested fields,

crop residues should be spread uniformly before planting to ensure optimal CE (Singh *et al.*, 2009b). As discussed earlier, multicrop new-generation ZT drills/planters, Turbo Happy Seeders, and rotary-disc drills allow seeding in loose and standing residues.

9.4.2. Chemical measures

Chemical control measures are generally more targeted at the early stage of weed emergence and growth when weed control is easier. Once weeds become big, they are difficult to control (Bastiaans *et al.*, 2008). In dry direct drill-seeded rice, the "critical period" of weed competition has been reported to be 15–45 days after seeding (Rao and Nagamani, 2007; Singh *et al.*, 1999; Yaduraju and Mishra, 2004). If weeds can be suppressed effectively during this period, minimal yield losses occur.

It is crucial to select the right herbicide depending upon the weed flora present in a given field. In addition, the correct rate, timing, and application techniques should be used. A variety of herbicides have been screened and found effective for preplant/burndown, preemergence, and postemergence weed control in dry direct drill-seeded rice systems, including under zero-tillage conditions. Table 16 provides an inventory of various available herbicides and their target weeds.

9.4.2.1. Preplant/burndown herbicides Preplant/burndown herbicides are used to control existing annual and perennial weeds prior to rice sowing, especially under the ZT system. Glyphosate (1 kg ai ha^{-1} or 0.5–1.0% by volume) and paraquat (0.5 kg ai ha^{-1} or 0.5% by volume) are recommended for burndown application (Gupta *et al.*, 2006). Glyphosate is a systemic nonselective herbicide, and it controls most annual and perennial weeds. To be effective, it should be applied when weeds are growing actively so that the herbicide is absorbed and translocated into the entire plant system. For the same reason, grazing of fields should be avoided. In a situation where the weeds are under stress, a light irrigation before spraying glyphosate is recommended. Paraquat is a nonselective contact herbicide, and it should be used in fields infested with annual weeds. This herbicide should be avoided in situations where fields are infested with perennial weeds. Clear water should be used for making a spray solution as these herbicides bind with suspended soil particles and metal surfaces (iron buckets), thereby reducing their efficiency (Gopal *et al.*, 2010). Nonreactive surfaces such as plastic containers should be used for preparing solutions. Moreover, the application of preplant herbicides is more effective when the weed foliage is fully exposed and is not submerged. If necessary, fields should be drained before application.

Table 16 Major herbicides used in direct-seeded rice and their target weed species

	Weed species	Preemergence		Postemergence									
		Pendimethalin	Oxadiargyl	Bispyribac	Penoxsulam	Fenoxaprop	Cyhalofop	Propanil	Azimsulfuron	Ethoxysulfuron	Triclopyr	2,4-D	Chlorimuron + metsulfuron
A.	Grass												
1	*Echinochloa crus-galli*	+	+	+	+	+	+	+	±	−	−	−	−
2	*E. colona*	+	+	+	+	+	+	+	±	−	−	−	−
3	*Leptochloa chinensis*	+	+	−	−	+	+	+	−	−	−	−	−
4	*Ergrostis japonica*	+	NA	−	−	+	+	+	NA	−	−	−	−
5	*Dactyloctenium aegyptium*	+	+	−	−	+	+	+	±	−	−	−	−
6	*Eleusine indica*	+	NA	−	±	+	NA	+	±	−	−	−	−
7	*Brachiaria reptans*	+	NA	−	−	+	+	+	NA	−	−	−	−
B.	Broadleaf												
8	*Eclipta alba*	+	+	+	+	−	−	+	+	+	+	+	+
9	*Caesulia axillaris*	NA	NA	NA	+	−	−	NA	+	NA	+	+	
10	*Sphenoclea zeylanica*	+	NA	+	+	−	−	±	+	NA	+	+	+
11	*Alternanthera sessile*	+	NA	NA	±	−	−	+		NA	+	+	
12	*Ammannia baccifera*	+	NA	NA	±	−	−	±	+	+	+	+	+
13	*Ludwigia quadrifolia*	+	+	+	±	−	−	±	+	NA		+	+
14	*Commelina* species	−	NA	+	+	−	−	NA	NA	NA	+	+	+
15	*Marsilea quadrifolia*	+	+	+	NA	−	−	NA	NA	+	NA	NA	+
16	*Monochoria*	NA	NA	+	+	−	−	±	NA	+		+	+
17	*Lindernia crustacea*	NA	NA	NA	+	−	−	+	+	NA	+	+	
18	*Trianthema portulacastrum*	+	NA	+	+	−	−	−	+	+	±	−	+
C.	Sedge												
19	*Cyperus iria*	+	+	+	+	−	−	+	+	+	+	±	+
20	*C. difformis*	+	+	+	+	−	−	+	+	+	±	±	+
21	*C. rotundus*	−	NA	±	±	−	−	−	+	+	NA	NA	±
22	*Fimbristylis miliacea*	+	NA	+	+	−	−	+	+	+	+	+	+

+, controlled; −, not controlled; ±, suppressed; NA, information not available.

9.4.2.2. Preemergence herbicides Pendimethalin (1.0 kg ai ha^{-1}), oxadiargyl (0.09 kg ai ha^{-1}), and pyrazosulfuron (0.02 kg ha^{-1}) have been reported to be effective preemergence herbicides to control weeds in dry direct-seeded rice (Gupta *et al.*, 2006; Rao and Nagamani, 2007, Singh *et al.*, 2009b; Gopal *et al.*, 2010). Good soil moisture is essential for the activation of preemergence herbicides. Pendimethalin should be applied after rice seed has imbibed germination water, that is, 2–3 days after sowing to avoid crop injury.

9.4.2.3. Postemergence herbicides Herbicides that have been found to be effective for postemergence weed control in the Dry-DSR system with their dose, time of application, mode of action, and strengths and weaknesses have been summarized in Table 17. Continuous use of a single herbicide on a long-term basis should be avoided; rather, it should be rotated with another herbicide with a different mode of action to avoid/delay resistance development. Tank mixtures of herbicides can be used when two or more herbicides are compatible to broaden the spectrum of weed control in such a way that each herbicide controls the weeds missed by the other one. The herbicide mixtures listed in Table 17 have been found to be effective in better controlling a combination of weeds, including grasses, broadleaves, and sedges.

9.4.3. Manual and mechanical methods of weed control

Relying only on manual weeding is not economical in most situations. One or two spot hand weedings may sometimes be necessary to remove weeds that have not been controlled by other weed control methods. For mechanical weeding, rotary weeders and cono weeders have been found effective in controlling weeds in DSR. More details on manual and mechanical methods of weed control can be obtained from reviews by Rao *et al.* (2007) and Singh *et al.* (2009b).

A well-leveled zero-tilled land coupled with a stale seedbed and residue mulch can be an effective method for suppressing weeds in Dry-DSR. Other cultural practices that help reduce weed pressure in Dry-DSR include the use of clean and certified seeds, keeping bunds and canals clean, good CE, varieties with greater weed-suppressive ability, and precise and proper water management (Singh *et al.*, 2009b).

In summary, the components of integrated strategies for weed control in DSR are (1) the stale seedbed technique, (2) the use of clean and certified seeds, (3) new herbicide chemistries appropriate to DSR conditions, (4) high-yielding rice varieties with greater early vigor and weed-competitive ability, (5) precise water management, (6) the use of mechanical tools and manual hand weeding, (7) the use of crop residues for weed suppression, and (8) the use of tillage practice (e.g., zero tillage), which provides habitat for seed predation and seed decay.

Table 17 Major pre- and postemergence herbicides used in direct-seeded rice in South Asia with application dose, timing, and their strengths and weaknesses

Herbicide	Dose (g ai ha^{-1})	Application time (DAS)[a]	Mode of action	Strengths	Weaknesses
Pendimethalin	1000	1–3	Microtubule assembly inhibitor	Good control of most grasses, some broadleaves and annual sedges. Has residual control	Sufficient moisture is needed for its activity
Oxadiargyl	90	1–3	Protoporphyrinogen oxidase inhibitor	Broad-spectrum weed control of grasses, broadleaves and annual sedges. Has residual control	Sufficient moisture is needed for its activity
Pyrazosulfuron	20	1–3 or 15–20 DAS	ALS inhibitor[b]	Broad-spectrum weed control of grasses, broadleaves and sedges including *C. rotundus*. Has residual control	Poor on grasses, including *L. Chinensis* and *Dactyloctenium aegyptium*

(*Continued*)

Table 17 (*Continued*)

Herbicide	Dose (g ai ha^{-1})	Application time (DAS)[a]	Mode of action	Strengths	Weaknesses
Bispyribac-sodium	25	15–25	ALS inhibitor	Broad-spectrum weed control of grasses, broadleaves and annual sedges. Excellent control of *Echinochloa* species	Poor on grasses other than *Echinochloa* species, including *L. chinensis*, *Dactyloctenium aegyptium*, *Eleusine indica*, *Ergrostis species*. No residual control
Penoxsulam	22.5	15–20	ALS inhibitor	Broad-spectrum weed control of grasses, broadleaves and annual sedges	Poor control of grasses other than *Echinochloa,* including *L. chinensis, D. aegyptium, Eleusine indica, Ergrostis species*
Fenoxaprop-ethyl	60	25	ACCase inhibitor[c]	Excellent control of annual grassy weeds	Does not control broadleaves and sedges. Not safe on rice if applied at early stage (before 25 DAS).

Fenoxaprop-ethyl + safner	60–90	15–20	ACCase inhibitor	Excellent control of annual grassy weeds, safe on rice at early stage	Does not control broadleaves and sedges
Cyhalofop-butyl	120	15–20	ACCase inhibitor	Excellent control of annual grassy weeds	Does not control broadleaves and sedges
Propanil	4000	15–25	Photosynthesis at photosystem-II inhibitor	Broad-spectrum weed control, can be tank-mixed with many herbicides	No residual control. Need sequential application for effective control or need some residual herbicide with it as tank mix
Azimsulfuron	17.5–35	15–20	ALS inhibitor	Broad-spectrum control of grasses, broadleaves and sedges. Excellent control of sedges, including *Cyperus rotundus*	Poor on *Echinochloa* species
Ethoxysulfuron	18	15–20	ALS inhibitor	Effective on broadleaves and annual sedges	Does not control grasses and poor on perennial sedges such as *C. rotundus*

(*Continued*)

Table 17 (*Continued*)

Herbicide	Dose (g ai ha^{-1})	Application time (DAS)[a]	Mode of action	Strengths	Weaknesses
Triclopyr	500	15–20	Synthetic auxins	Effective on broadleaf weeds	Does not control grasses
2,4-D ethyl ester	500	15–25	Synthetic auxins	Effective on broadleaves and annual sedges. Very economical	Has no residual control
Carfentrazone	20	15–20		Effective on broadleaf weeds	Does not control grasses. Has no residual control
Chlorimuron + metsulfuron	4 (2 + 2)	15–25	ALS inhibitor	Effective on broadleaves and annual sedges	No control of grassy weeds and poor on *C. rotundus*
Bispyribac + azimsulfuron	25 + 17.5	15–25	ALS inhibitor	Broad-spectrum weed control of grasses, broadleaves and sedges, including *C. rotundus*	Poor on grasses other than *Echinochloa* species

Fenoxaprop + ethoxysulfuron	56 + 18	15–25	ACCase and ALS	Broad-spectrum weed control of grasses, broadleaves and sedge. Excellent control of all major grasses, including *L. chinensis* and *D. aegyptium*	Poor on perennial sedges such as *C. rotundus*
Propanil + pendimethalin	4000 + 1000	10–12 DAS	Photosynthesis and microtubule assembly inhibitor	Broad-spectrum weed control with residual effects	Poor on sedges such as *C. rotundus*
Propanil + triclopyr	3000 + 500	15–25	Photosynthesis and synthetic auxins	Broad-spectrum weed control of grasses, broadleaves and sedges	Poor control on perennial sedges such as *C. rotundus*. No residual control

[a] Days after sowing.
[b] Acetolactate synthesis inhibitor.
[c] Acetyl-coenzyme A carboxylase synthesis inhibitor.

9.5. Fertilizer management

Much work on fertilizer management in rice has been carried out for CT-TPR but limited work has been conducted in Dry-DSR, including ZT-dry-DSR. In Dry-drill-DSR, because of more aerobic conditions and alternate wetting/drying cycles, the availability of several nutrients including N and micronutrients such as Zn and Fe, is likely to be a constraint (Ponnamperuma, 1972). In addition, loss of N due to nitrification/denitrification, volatilization, and leaching is likely to be higher in Dry-DSR than in CT-TPR (Musa 1969; Davidson, 1991; Singh and Singh, 1988; Patrick and Wyatt, 1964).

General recommendations for NPK fertilizers are similar to those in puddled transplanted rice, except that a slightly higher dose of N (22.5–30 kg ha^{-1}) is suggested in DSR (Dingkuhn *et al*., 1991a; Gathala *et al*., 2011). This is to compensate for the higher losses and lower availability of N from soil mineralization at the early stage as well as the longer duration of the crop in the main field in Dry-DSR. Early studies conducted in Korea indicated that 40–50% more N fertilizer should be applied in Dry-DSR than in CT-TPR (Park *et al*., 1990; Yun *et al*., 1993), although higher N application also leads to disease susceptibility and crop lodging. The general recommendation is to apply a full dose of P and K and one-third N as basal at the time of sowing using a seed-cum-fertilizer drill/planter. This allows placement of the fertilizer just below the seeds and hence improves fertilizer efficiency. Split applications of N are necessary to maximize grain yield and to reduce N losses and increase N uptake. Split applications ensure a supply of N to match crop demand at the critical growth stages. The remaining two-third dose of N should be applied as topdressing in equal parts at active tillering and panicle initiation stage. In addition, N can be managed using a leaf color chart (LCC) (Shukla *et al*., 2004; Alam *et al*., 2005). Two options are recommended for applying fertilizer N using an LCC (IRRI, 2010). In the fixed-time option, N is applied at a preset timing of active tillering and panicle initiation, and the dose can be adjusted upward or downward based on leaf color. In the real-time option, farmers monitor the color of rice leaves at regular intervals of 7–10 days from early tillering (20 DAS) and N is applied whenever the color is below a critical threshold value (IRRI, 2010). For high-yielding inbreds and hybrids, N application should be based on a critical LCC value of 4, whereas, for basmati types, N should be applied at a critical value of 3 (Shukla *et al*., 2004; Gupta *et al*., 2006; Gopal *et al*., 2010). Since more N is applied in Dry-DSR and losses are higher than in CT-TPR, more efficient N management for Dry-DSR is needed.

Slow-release (SRF) or controlled-release N fertilizers (CRFs) offer the advantage of a "one-shot dose" of N and the option to reduce N losses because of their delayed release pattern, which may better match crop demand (Shoji *et al*., 2001). One-shot application will also reduce labor

cost. Fashola *et al.* (2002) reported that CRF improves N use efficiency and yield compared with untreated urea. Because of these benefits, CRF with polymer-coated urea is used by Japanese farmers in ZT-dry-DSR (Saigusa, 2005; Ando *et al.*, 2000). Despite these benefits, farmers' use of CRF is limited mainly because of the high costs associated with it. The cost of CRF may be four to eight times higher than that of conventional fertilizers (Shaviv and Mikkelsen, 1993). In addition, published results on the performance of SRFs/CRFs compared with conventional fertilizers are not consistent. Christianson and Schultz (1991), Stangel *et al.* (1991), Stutterheim *et al.* (1994), and Fashola *et al.* (2002) have demonstrated higher N use efficiency through the use of CRFs. Saigusa (2005) reported higher N recovery of co-situs (placement of both fertilizer and seeds or roots at the same site) application of CRF with polyolefin-coated ureas of 100-day type (POCU-100) than conventional ammonium sulfate fertilizer applied as basal and topdressed in zero-till direct-seeded rice in Japan. In contrast, Wilson *et al.* (1990), Wells and Norman (1992), and Golden *et al.* (2009) reported inferior performance of SRF or CRF compared with conventional urea topdressed immediately before permanent flood establishment.

Split application of K has also been suggested for direct seeding in medium-textured soil (PhilRice, 2002). In these soils, K can be split, with 50% as basal and 50% at early panicle initiation stage. Deficiency of Zn and Fe is more common in aerobic/non-flooded rice systems than in flooded rice systems (Sharma *et al.*, 2002; Singh *et al.*, 2002a; Hongbin *et al.*, 2006; Choudhury *et al.*, 2007; Pal *et al.*, 2008; Yadvinder-Singh *et al.*, 2008). Therefore, micronutrient management is critical in Dry-DSR. To avoid zinc deficiency, 25–50 kg ha^{-1} zinc sulfate is recommended (Anonymous, 2008, 2010). Basal application of zinc to the soil is found to be the best. However, if a basal application is missed, the deficiency can be corrected by topdressing up to 45 days (Anonymous, 2010). Zinc can be supplied by foliar application (0.5% zinc sulfate) two to three times at intervals of 7–15 days just after the appearance of deficiency symptoms. For iron, it has been observed that foliar application is superior to soil application (Datta *et al.*, 2003; Anonymous, 2010). Foliar-applied Fe is easily translocated acropetally and even retranslocated basipetally. A total of 9 kg Fe ha^{-1} in three splits (40, 60, and 75 DAS) as foliar application (3% of $FeSO_4 \cdot 7H_2O$ solution) has been found to be effective (Pal *et al.*, 2008).

10. Conclusions and Future Outlook

Today, conventional puddled transplanting is the most common practice of rice production in Asia. Because of the water-, labor-, and energy-intensive nature of this system, and rising interest in CA, dry-seeded rice (Dry-DSR) with zero or reduced tillage (ZT–RT) has emerged as a viable

alternative. Projections and trends seem to suggest that Dry-DSR will likely be a major rice culture in many countries in the future. We have attempted to address several questions pertaining to DSR and discuss an integrated package of technologies specifically for ZT/RT-dry-DSR to address the fast-emerging water and labor crisis.

10.1. What are the different types of direct seeding and their niches?

Crop establishment, though using direct sowing, can vary from broadcasting manually or mechanically (aeroplane or power sprayer) to line sowing using either a drill or a drum seeder, or manually by the dibble method in puddled or unpuddled soil. In areas where labor scarcity has been serious but water is relatively more readily available, farmers shifted to Wet-DSR without making a change in tillage. However, in areas where both labor and water are emerging as major constraints, farmers are interested in Dry-DSR with zero or reduced tillage.

10.2. What are the major drivers of the shift from puddled transplanting to direct seeding?

The rising scarcity of water and labor are the major drivers for this shift. Puddled transplanting is the main user of freshwater, and it requires large amounts of labor. However, water and labor for agriculture are becoming increasingly scarce resources in many rice production areas. The share of water in agriculture is declining because of its increased demand in other nonagriculture sectors. Groundwater is being depleted at an alarming rate, especially in South Asia and North China, mainly because of its heavy use for rice production. Similarly, labor availability for agriculture is declining because of increased demand in nonagriculture sectors associated with rapid economic growth in many Asian countries. Moreover, in the current socioeconomic environment, most people, especially young workers, are unwilling to undertake tedious farm operations such as transplanting. In addition, high labor demand during the critical operation of transplanting leads to shortages and increasing labor costs. These factors provide incentives for farmers to shift to some form of direct seeding, which requires less water and labor. Other drivers include (1) economic incentives for crop intensification (from a single rice crop to double cropping in Vietnam and the Philippines) brought by DSR, (2) the adverse effect of puddling on physical properties of the soil and on the succeeding non-rice upland crop in rotation together with rising interest in CA, and (3) recent developments in production techniques along with the availability of new herbicides for weed control and short-duration varieties. Primarily because of labor shortages, direct seeding (mostly wet seeding) is widely adopted in Malaysia and Sri Lanka and is spreading rapidly in Vietnam, Thailand, and the Philippines.

10.3. What lessons have we learned from those countries where direct seeding is widely adopted?

In the United States, Malaysia, and Sri Lanka, more than 90% of the rice has been direct seeded for the past few decades. These case studies provide a few important lessons for the countries that are moving toward DSR. It is clear that precise land leveling, suitable cultivars, good CE, precise water management, and effective and efficient weed and nutrient management are keys to the success of DSR. The establishment of a strong herbicide industry resulting in the availability of affordable and appropriate herbicides has also played an important role in these countries. Experiences have also shown that a shift to DSR resulted in (1) weed flora changes toward more difficult-to-control and competitive grasses and sedges, (2) the development of resistance in weeds against commonly used herbicides, and (3) the appearance of weedy rice. Therefore, anticipatory research and development strategies need to be developed for areas where direct seeding is likely to be adopted. This is important for direct seeding to be sustainable on a long-term basis.

10.4. Can direct seeding be as productive as conventional puddled transplanted rice?

Available published data in the literature show variable responses of crop productivity under DSR. Wet-DSR with line sowing (CT-wet-DSR) tends to be as good as or superior to CT-TPR. Dry-DSR has been more inconsistent, with a yield penalty ranging from 7.5% to 28.5% in India and Pakistan, whereas in other countries, it was similar to CT-TPR. However, the gradual improvement in productivity observed in Wet-DSR is likely to also occur for the more recently introduced Dry-DSR systems as optimal complementary management practices are developed.

10.5. Does direct seeding save on the use of labor or water?

Published data from 44 studies show clear evidence of savings of 12–35% of irrigation water under DSR systems. Irrigation water savings ranked in the following order: Bed-dry-DSR > ZT-dry-DSR > CT-dry-DSR > CT-wet-DSR > CT-TPR. The irrigation water productivity of DSR methods was either similar (ZT-dry-DSR and Bed-dry-DSR) or higher (CT-wet-DSR and CT-dry-DSR) than in CT-TPR. Labor savings of up to 60% in DSR compared with CT-TPR have been reported, with the level of savings depending on tillage, CE method, and level of mechanization. ZT-Dry-DSR saves more labor than Wet-DSR because of additional savings in land preparation.

10.6. Is direct-seeded rice economically attractive to farmers?

Farmers have perfected puddling and transplanting over time and are reluctant to try alternatives. However, economics play an important role in the decision making of farmers. Trials that are largely conducted by researchers clearly show economic advantages in DSR over puddled transplanting. Overall, based on 77 studies, DSR compared with CT-TPR had a lower cost of production by US\$22–80 ha^{-1} and savings in production costs increased in the following order: ZT-dry-DSR > Bed-dry-DSR > CT-dry-DSR ≥ CT-wet-DSR > CT-TPR. Overall, except for Bed-dry-DSR, all DSR methods resulted in US\$30–50 ha^{-1} higher economic returns than CT-TPR, but with a lower cost of production.

10.7. How does direct-seeded rice influence greenhouse gas emissions?

Well-managed studies demonstrate reductions in methane emissions in DSR (8–92%) compared with CT-TPR, with the greatest reductions occurring in Dry-DSR. These reductions in methane emissions are largely due to the avoidance of standing water in fields with direct seeding. However, several studies suggest two- to sixfold increases in N_2O emissions when shifting to direct seeding, especially with ZT-dry-DSR. A complete picture of the influence of alternative tillage and CE practices on three GHGs (CH_4, N_2O, and CO_2) in terms of GWP is lacking. It is expected that changes in tillage, especially residue, water, and N management, will have a significant impact on all the GHGs. Baseline data are urgently needed to develop improved management practices that are more environmentally friendly. Limited studies indicate that ZT-dry-DSR compared with CT-TPR has potential to reduce GWP by 20–44%.

10.8. What plant traits are the most important for optimizing direct-seeding systems?

Relatively little work has targeted selection and breeding of rice for direct seeding, especially under zero tillage in Asia. Generally, rice varieties bred for puddled transplanting are used in direct seeding. The lack of suitable varieties is a major constraint to achieving maximum potential of direct seeding. The traits that are likely to be most helpful for direct seeding include (1) anaerobic seed germination and tolerance of early submergence for quick CE, (2) high seedling vigor with faster leaf area development (semierect leaves with high specific leaf area) during the early vegetative stage for weed suppression, (3) erect leaves with low specific leaf area and high chlorophyll content for high crop growth during the reproductive phase along with high remobilization ability for higher spikelet fertility, (4) a

strong, thick, and sturdy culm with long and heavy panicles positioned at lower height for lodging resistance, and (5) high genetic yield potential with high input use efficiency under DSR.

10.9. What have we achieved and what is still needed for attaining maximum potential of direct-seeded rice?

Realizations that (a) optimal plant architecture in DSR could be critical to the success of DSR, just as it has been for puddled transplanting, and (b) the importance of rapid emergence and subsequent good establishment during the early stage of rice growth have been important developments in our thinking process. This has led to the development of management practices that enhance stand establishment, including land leveling, seeding (depth, density, distance) with residue, irrigation, and weed control. Seeding at an optimal depth and distance has not only reduced the seed rate from 80–200 kg ha^{-1} to around 25 kg ha^{-1} but has also helped in overcoming spikelet sterility and lodging problems. Both agronomic management and a suitable variety with appropriate traits are needed to achieve maximum potential under DSR. Much research and many adoptive evaluations carried out during the past decade have provided management options, including improved drills to precisely place seed and fertilizer. We are making good progress in managing weeds using integrated approaches. However, additional research is needed in weed management, including (1) monitoring shifts in weed flora, (2) developing management strategies for emerging problems of weedy rice, (3) identifying new herbicides/tank mixtures with wide-spectrum weed control ability, (4) identifying vulnerabilities in weed life cycles through analysis of weed population dynamics under reduced till/ZT conditions, and (5) developing integrated strategies to minimize/avoid/delay the development of herbicide resistance in weed populations. Although refinements in agronomy and management will continue to be important, targeting varietal improvements in rice under DSR is likely to crucial for improving the potential of direct seeding.

ACKNOWLEDGMENTS

We are grateful to Drs. Daniel Brainard, Michigan State University; Andrew McDonald, CIMMYT-Nepal; A. N. Rao, IRRI-India; Elizabeth Humphreys, IRRI-Philippines for providing critical and constructive comments on this chapter; and Dr. Bill Hardy, Senior editor, IRRI for editing the chapter.

REFERENCES

Adisarwanto, T., Santoso, B., Manshuri, G., and Floyd, C. (1989). *In* "Agronomic survey of MK1 soybean on sawah in East Java (Jember to Madiun)", 21–24 June 1989, Malang Research Institute for Food Crops, E. Java, Indonesia.

Aggarwal, G. C., Sidhu, A. S., Sekhon, N. K., Sandhu, K. S., and Sur, H. S. (1995). Puddling and N management effects on crop response in a rice-wheat cropping system. *Soil Till. Res.* **36,** 129–139.

Ahmed, K. M., Bhattacharya, P., Hasan, M. A., Akhter, S. H., Alam, S. M. M., Bhuyian, M. A. H., Imam, M. B., Khan, A. A., and Sracek, O. (2004). Arsenic enrichment in groundwater of the alluvial aquifers in Bangladesh: An overview. *Appl. Geochem.* **19,** 181–200.

Alam, M. M., Ladha, J. K., Khan, R. S., Foyjunnessa, Rashid H., Khan, A. H., and Buresh, R. J. (2005). Leaf color chart for managing nitrogen fertilizer in lowland rice in Bangladesh. *Agron. J.* **97,** 949–959.

Ando, H., Kakuda, K., Nakayama, M., and Yokoto, K. (2000). Yield of no-tillage direct-seeded lowland rice as influenced by different sources and application methods of fertilizer nitrogen. *Soil Sci. Plant Nutr.* **46,** 105–115.

Anonymous (2008). Package and Practices of *Kharif* Crops. CCS Haryana Agricultural University, Hisar, Haryana.

Anonymous. (2010). Micronutrient deficiencies in Indian soils and field usable practices for their correction Indian Institute of Soil Science, Bhopal, www.iiss.nic.in/Recommendations1.pdf, (accessed June 9, 2010).

Arora, V. K., Gajri, P. R., and Uppal, H. S. (2006). Puddling, irrigation, and transplanting-time effects on productivity of rice–wheat system on a sandy loam soil of Punjab. *India. Soil Till. Res.* **85,** 212–220.

Audebert, A., Dingkuhn, M., Jones, M. P., and Johnson, D. E. (1999). Physiological mechanisms for vegetative vigor of interspecific upland rice—implications for weed competitiveness. "Proceedings of the International Symposium on World Food Security", pp. 300–301, Kyoto.

Aulakh, M. S., Wassmann, R., and Rennenberg, H. (2001). Methane emissions from rice fields: Quantification, mechanisms, role of management, and mitigation options. *Adv. Agron.* **70,** 93–260.

Azmi, M., and Abdullah, M. Z. (1998). A Manual for the Identification and Control of Padi Angin (Weedy Rice) in Malaysia. MARDI Publication, Serdang, Malaysia, 18p.

Azmi, M., and Baki, B. B. (2007). Weed flora landscapes of the Muda rice granary in the new millennium: A descriptive analysis. *J. Trop. Agric. Food Sci.* **35,** 319–332.

Azmi, M., and Johnson, D. E. (2009). Crop establishment options for lowland irrigated rice in relation to weed infestation and grain yield. *J. Trop. Agric. Food Sci.* **37,** 111–117.

Azmi, M., and Mashor, M. (1995). Weed succession from transplanting to direct-seeding method in Kemubu rice area. *Malaysia J. Biosci.* **6,** 143–154.

Azmi, M., Baki, B. B., and Mashhor, M. (1993). Weed communities in principal rice growing areas in Peninsular Malaysia. MARDI Report No. 165, MARDI, Kuala Lumpur, Malaysia, pp. 1–16.

Azmi, M., Chin, D. V., Vongsaroj, P., and Johnson, D. E. (2005). Emerging issues in weed management of direct-seeded rice in Malaysia, Vietnam, and Thailand. *In* "Rice Is Life: Scientific Perspectives for the 21st Century" (K. Toriyama, K. L. Heong, and B. Hardy, Eds.), pp. 196–198. International Rice Research Institute, Los Baños, Philippines and Japan International Research Center for Agricultural Sciences, Tsukuba, Japan.

Bajpai, R. K., and Tripathi, R. P. (2000). Evaluation of non-puddling under shallow water tables and alternative tillage methods on soil and crop parameters in a rice–wheat system in Uttar Pradesh. *Soil Till. Res.* **55,** 99–106.

Bakar, B. B., Bakar, M. A., and Man, A. B. (2000). Weedy rice (*Oryza sativa* L.) in Peninsular Malaysia. *In* "Wild and Weedy Rice in Rice Ecosystems in Asia: A Review" (B. B. Baki, D. V. Chin, and M. Mortimer, Eds.), pp. 51–54. International Rice Research Institute, Los Baños, PhilippinesLimited Proceedings No. 2.

Balasubramanian, V., and Hill, J. E. (2002). Direct seeding of rice in Asia: Emerging issues and strategic research needs for the 21st century. *In* "Direct Seeding: Research Strategies and Opportunities" (S. Pandey, M. Mortimer, L. Wade, T. P. Tuong, K. Lopez, and B. Hardy, Eds.), pp. 15–39. International Rice Research Institute, Los Baños, Philippines.

Baloch, M. S., Awan, I. U., Hasa, G., and Zubair, M. (2007). Studies on plant population and stand establishment techniques for increasing productivity of rice on Dera Ismail Khan. *Pakistan Rice Sci.* **14,** 118–224.

Baltazar, A. M., and De Datta, S. K. (1992). Weed management in rice. *Weed Abstr.* **41,** 495–508.

Barker, R., Dawe, D., Tuong, T. P., Bhuiyan, S. I., and Guerra, L. C. (1998). The outlook for water resources in the year 2020: Challenges for research on water management in rice production. *In* "Assessment and Orientation Towards the 21st Century". 7–9 September 1998. Proceedings of 19th Session of the International Rice Commission, Cairo, Egypt, FAO, pp. 96–109.

Bastiaans, L., Paolini, R., and Baumann, D. T. (2008). Focus on ecological weed management: What is hindering adoption? *Weed Res.* **48,** 481–491.

Bazaya, B. R., Sen, A., and Srivastava, V. K. (2009). Planting methods and nitrogen effects on crop yield and soil quality under direct seeded rice in the Indo-Gangetic plains of eastern India. *Soil Till. Res.* **105,** 27–32.

Begum, M., Juraimi, A. S., Azmi, M., Rajan, A., and SyedOmar, S. R. (2005). Weed vegetation of direct seeded rice fields in Muda rice granary areas of Peninsular Malaysia. *Pakistan J. Biol. Sci.* **8,** 537–541.

Bhattacharjee, I. R. (1978). Note on the seed rate as cultural method of weed control for direct seeding short duration paddy (Pusa 2–21). *Sci. Culture* **44,** 504–505.

Bhuiyan, S. I., Sattar, M. A., and Khan, M. A. K. (1995). Improving water use efficiency in rice irrigation through wet seeding. *Irrig. Sci.* **16,** 1–8.

Bhushan, Lav, Ladha, J. K., Gupta, R. K., Singh, S., Tirol-Padre, A., Saharawat, Y. S., Gathala, M., and Pathak, H. (2007). Saving of water and labor in rice-wheat systems with no-tillage and direct seeding technologies. *Agron. J.* **99,** 1288–1296.

Blanche, B., Harrell, D., and Saichuk, J. (2009). General Agronomic Guidelines. *In* "Louisiana rice production handbook 2009" (J. Saichuk, Ed.), pp. 3–15, Pub. 2321 (3M) 6/09 Rev.

Boparai, B. S., Singh, Y., and Sharma, B. D. (1992). Effect of green manuring with *Sesbania aculeata* on physical properties of soil and on growth of wheat in rice–wheat and maize–wheat cropping systems in a semiarid region of India. *Arid Soil Res. Rehab.* **6,** 135–143.

Bouman, B. A. M. (2009). How much water does rice use? *Rice Today* **8,** 28–29.

Bouman, B. A. M., and Tuong, T. P. (2001). Field water management to save water and increase its productivity in irrigated rice. *Agric. Water Manage.* **49,** 11–30.

Bouman, B. A. M., Lampayan, R. M., and Tuong, T. P. (2007). Water Management in Irrigated Rice: Coping with Water Scarcity. International Rice Research Institute, Los Baños, Philippines, 54p.

Bouman, B. A. M., Peng, S., Castañeda, A. R., and Visperas, R. M. (2005). Yield and water use of irrigated tropical aerobic rice systems. *Agric. Water Manage.* **74,** 87–105.

Cabangon, R. J., Tuong, T. P., and Abdullah, N. B. (2002). Comparing water input and water productivity of transplanted and direct-seeded rice production systems. *Agric. Water Manage.* **57,** 11–31.

Cabangon, R. J., Tuong, T. P., Castillo, E. G., Bao, L. X., Lu, G., Wang, G. H., Cui, L., Bouman, B. A. M., Li, Y., Chen, C., and Wang, J. (2004). Effect of irrigation method and N-fertilizer management on rice yield, water productivity and nutrient-use efficiencies in typical lowland rice conditions in China. *Paddy Water Environ.* **2,** 195–206.

Can, N. D., and Xuan, V. T. (2002). Environmental conditions as determinants of direct seeding techniques in different ecosystems in the Mekong Delta of Vietnam. *In* "Proceedings of the International Workshop on Direct Seeding in Asian Rice Systems: Strategic Research Issues and Opportunities" (S. Pandey, M. Mortimer, L. Wade, T. P. Tuong, K. Lopez, and B. Hardy, Eds.), pp. 75–86. International Rice Research Institute, Los Baños, Philippines.

Carriger, S., and Vallée, D. (2007). More crop per drop. *Rice Today* **6**(2), 10–13.

Cassman, K. G. (1999). Ecological intensification of cereal production systems: Yield potential, soil quality, and precision agriculture. *Proc. Natl. Acad. Sci. USA* **96,** 5952–5959.

Castin, E. M., and Moody, K. (1989). Effect of different seeding rates, moisture regimes, and weed control treatments on weed growth and yield of wet-seeded rice. *In* "Proceedings of the 12th Asian-Pacific Weed Science Society Conference, Seoul, Korea", pp. 337–343.

Caton, B. P., Cope, A. E., and Mortimer, M. (2003). Growth traits of diverse rice cultivars under severe competition: Implications for screening for competitiveness. *Field Crops Res.* **83,** 157–172.

Chauhan, B. S., and Johnson, D. E. (2008a). Germination ecology of Chinese sprangletop (*Leptochloa chinensis*) in the Philippines. *Weed Sci.* **56,** 820–825.

Chauhan, B. S., and Johnson, D. E. (2008b). Influence of environmental factors on seed germination and seedling emergence of eclipta (*Eclipta prostrata*) in a tropical environment. *Weed Sci.* **56,** 383–388.

Chauhan, B. S., and Johnson, D. E. (2009). Ecological studies on *Cyperus difformis*, *C. iria* and Fimbristylis miliacea: Three troublesome annual sedge weeds of rice. *Ann. Appl. Biol.* **155,** 103–112.

Chauhan, B. S., and Johnson, D. E. (2010). The role of seed ecology in improving weed management strategies in the tropics. *Adv. Agron.* **105,** 221–262.

Chauhan, B. S., Gill, G., and Preston, C. (2006). Tillage system effects on weed ecology, herbicide activity and persistence: A review. *Aust. J. Exp. Agric.* **46,** 1557–1570.

Chavez, R. S. C. (1989). Cultivar competitiveness and weed control in upland rice. M.Sc. Thesis. University of Philippines, Los Baños, College, Laguna, Philippines.

Chenkual, V., and Acharya, C. L. (1990). Effect of rice–wheat and maize–wheat rotations on soil physical properties including soil–water behavior in an acidic Alfisol. *J. Indian Soc. Soil Sci.* **38,** 574–582.

Chin, D. V. (1997). Occurrence of weedy rice in Vietnam. *In* "Proceedings of the 16th Asian–Pacific Weed Science Society Conference", Asian–Pacific Weed Science Society, Kuala Lumpur, Malaysia.

Chin, D. V. (2001). Biology and management of barnyardgrass, red sprangletop and weedy rice. *Weed Biol. Manage.* **1,** 37–41.

Chin, D. V., Hien, T. V., and Thiet, L. V. (2000). Weedy rice in Vietnam. *In* "Wild and Weedy Rice in Rice Ecosystems in Asia: A Review" (B. B. Baki, D. V. Chin, and M. Mortimer, Eds.), pp. 45–50. Limited Proceedings No. 2. International Rice Research Institute, Los Baños, Philippines.

Chou, C. H. (1999). Roles of allelopathy in plant biodiversity and sustainable agriculture. *Crit. Rev. Plant Sci.* **18,** 609–636.

Choudhury, B. U., Bouman, B. A. M., and Singh, A. K. (2007). Yield and water productivity of rice-wheat on raised beds at New Delhi. *India Field Crops Res.* **100,** 229–239.

Christianson, C. B., and Schultz, J. J. (1991). Strategies to improve nitrogen fertilizer use efficiency in upland systems. *In* "Paper Presented at Training Program: Plant Nutrient Management for Sustainable Agriculture," October 1991. IFDC, Muscle Shoals, Alabama.

Corton, T. M., Bajita, J., Grospe, F., Pamplona, R., Wassmann, R., and Lantin, R. S. (2000). Methane emission from irrigated and intensively managed rice fields in Central Luzon (Philippines). *Nutr. Cycl. Agroecosyst.* **58,** 37–53.

Cousens, R. D. (1996). Comparative growth of wheat, barley, and annual ryegrass (*Lolium rigidum*) in monoculture and mixture. *Aust. J. Agric. Res.* **47,** 449–464.

Cui, K., Peng, S., Xing, Y., Xu, C., Yu, S., and Zhang, Q. (2002). Molecular dissection of seedling-vigor and associated physiological traits in rice. *Theor. Appl. Genet.* **105,** 745–753.

Datta, S. P., Saharan, N., and Rattan, R. K. (2003). Micronutrient fertilizers, sources and methods of application. *In* "Integrated Micronutrient Management for Sustainable Agriculture" (D. K. Das and D. Saha, Eds.), pp. 71–89. BCKV, West Bengal, India.

Davidson, E. A. (1991). Fluxes of nitrous oxide and nitric oxide from terrestrial ecosystems. *In* "Microbial Production And Consumption Of Greenhouse Gases: Methane, Nitrogen Oxides and Halomethanes" (J. E. Rogers and W. B. Whitman, Eds.), pp. 219–235. The American Society of Microbiology, Washington, DC.

Dawe, D., Frolking, S., and Li, C. (2004). Trends in rice-wheat area in China. *Field Crops Res.* **87,** 89–95.

Dawe, D. (2005). Increasing water productivity in rice-based systems in Asia—Past trends, current problems, and future prospects. *Plant Prod. Sci.* **8,** 221–230.

De Datta, S. K. (1981). Principles and Practices of Rice Production. John Wiley and Sons, New York, 618pp.

de Dios, J. L., Javier, E. F., Malabayabas, M. D., Casimero, M. C., and Espiritu, A. J. (2005). An overview on direct-seeding for rice crop establishment in the Philippines. *In* "Rice Is Life: Scientific Perspectives for the 21st Century" (K. Toriyama, K. L. Heong, and B. Hardy, Eds.), pp. 189–193. International Rice Research Institute/Japan International Research Center for Agricultural Sciences, Los Baños, Philippines/Tsukuba, Japan, CD.

Deb Roy, A., and Shah, T. (2002). The socio-ecology of groundwater in India. Water Policy Briefing No. 4. *In* "International Water Management Institute." Colombo, Sri Lanka.

Dhiman, S. D., Sharma, H. C., Nandal, D. P., Om, H., and Singh, D. (1998). Effect of irrigation, methods of crop establishment and fertilizer management on soil properties and productivity in rice (*Oryza sativa*)-wheat (*Triticum aestivum*) sequence. *Indian J. Agron.* **43,** 208–212.

Diarra, A., Smith, R. J. Jr., and Talbert, R. E. (1985). Interference of red rice (*Oryza sativa*) with rice (*O. sativa*). *Weed Sci.* **33,** 644–649.

Dilday, R. H., Mgonja, M. A., Amonsilpa, S. A., Collins, F. C., and Well, B. R. (1990). Plant height vs. mesocotyl and coleoptile elongation in rice: Linkage or pleiotropism? *Crop Sci.* **30,** 815–818.

Dingkuhn, M., De Datta, S. K., Pamplona, R., Javellana, C., and Schnier, H. F. (1992). Effect of late-season N fertilization on photosynthesis and yield of transplanted and direct-seeded tropical flooded rice. II. A canopy stratification study. *Field Crops Res.* **28,** 235–249.

Dingkuhn, M., Penning de Vries, F. W. T., De Datta, S. K., and van Laar, H. H. (1991a). Concepts for a new plant type for direct seeded flooded tropical rice. *In* "Direct Seeded Flooded Rice in the Tropics", pp. 17–38. International Rice Research Institute (IRRI), Los Baños, Philippines.

Dingkuhn, M., Johnson, D. E., Sow, A., and Audebert, A. Y. (1999). Relationships between upland rice canopy characteristics and weed competitiveness. *Field Crops Res.* **61,** 79–95.

Dingkuhn, M., Schnier, H. F., De Datta, S. K., Dorffling, K., and Javellana, C. (1990). Nitrogen fertilization of direct-seeded flooded vs. transplanted rice. II. Interactions among canopy properties. *Crop Sci.* **30,** 1284–1292.

Dingkuhn, M., Schnier, H. F., De Datta, S. K., Dorffling, K., and Javellana, C. (1991b). Relationships between ripening-phase productivity and crop duration, canopy photosynthesis and senescence in transplanted and direct-seeded lowland rice. *Field Crops Res.* **26,** 327–345.

Dofing, S. M., and Knight, C. W. (1994). Yield component compensation in uniculm barley lines. *Agron. J.* **86,** 273–276.

Dong, B., Molden, D., Loeve, R., Li, Y. H., Chen, C. D., and Wang, J. Z. (2004). Farm level practices and water productivity in Zanghe Irrigation System. *Paddy Water Environ.* **2,** 217–226.

Eleftherohorinos, I. G., Dhima, K. V., and Vasilakoglou, I. B. (2002). Interference of red rice in rice grown in Greece. *Weed Sci.* **50,** 167–172.

Erenstein, O., and Laxmi, V. (2008). Zero tillage impact in India's rice-wheat systems: A review. *Soil Till. Res.* **100,** 1–14.

FAO AQUASTAT. (2009). FAOSTAT Database on Water and Agriculture FAO, Rome, www.fao.org/nr/water/aquastat/data/query/index.html?lang=en (accessed June 2010).

FAO-Food and Agriculture Organization (1999). *In* "Report of Global Workshop on Red Rice Control, August 30 to September 3, 1999, Varadero, Cuba". FAO, Rome p. 155.

FAO-Food and Agriculture Organization (2009). FAOSTAT Database FAO, Rome, www.faostat.fao.org (accessed June 2010).

FAO-Food and Agriculture Organization (2010). *In* "FAOSTAT Database". FAO, Rome, www.faostat.fao.org (accessed June 2010).

Farooq, M., Basra, S. M. A., and Asad, S. A. (2008). Comparison of conventional puddling and dry tillage in rice–wheat system. *Paddy Water Environ.* **6,** 397–404.

Farooq, M., Basra, S. M. A., and Wahid, A. (2006a). Priming of field-sown rice seed enhances germination, seedling establishment, allometry and yield. *Plant Growth Regul.* **49,** 285–294.

Farooq, M., Basra, S. M. A., Tabassum, R., and Afzal, I. (2006b). Enhancing the performance of direct seeded fine rice by seed priming. *Plant Prod. Sci.* **9,** 446–456.

Fashola, O. O., Hayashi, K., and Wakatsuki, T. (2002). Effect of water management and polyolefin-coated urea on growth and nitrogen uptake of indica rice. *J. Plant Nutr.* **25,** 2173–2190.

Ferrero, A. (2003). Weedy rice, biological features and control. *In* "Weed Management for Developing Countries: Addendum 1" (R. Labrada, Ed.), pp. 89–107. FAO, Rome FAO Plant Production and Protection Paper 120, Add. 1.

Fischer, A. J., Ramirez, H. V., and Lozano, J. (1997). Suppression of junglerice (*Echinochloa colona* (L.) Link) by irrigated rice cultivars in Latin America. *Agron. J.* **89,** 516–552.

Fischer, A. J., Ramirez, H. V., Gibson, K. D., and Pinheiro, B. D. S. (2001). Competitiveness of semidwarf upland rice cultivars against palisadegrass (*Brachiaria brizantha*) and signalgrass (*B. decumbens*). *Agron. J.* **93,** 967–973.

Fuji, H., and Cho, M. C. (1996). Water management under direct seeding. *In* "Recent Advances in Malaysian Rice Production" (Y. Morrka, S. Jegatheesan, and K. Yasunobu, Eds.), pp. 113–129. Integrated Study Report, MADA/JIRCAS, Malaysia.

Fukai, S. (2002). Rice cultivar requirement for direct-seeding in rainfed lowlands. *In* "Direct seeding: Research strategies and opportunities". (S. Pandey, M. Mortimer, L. Wade, T. P. Tuong, K. Lopez, and B. Hardy, Eds.), Proceedings of the International Workshop on Direct Seeding in Asian Rice Systems: Strategic Research Issues and Opportunities, 25–28, January 2000, pp. 15–39.

Gajri, P. R., Arora, V. K., and Prihar, S. S. (1992). Tillage management for efficient water and nitrogen use in wheat following rice. *Soil Till. Res.* **24,** 167–182.

Gajri, P. R., Gill, K. S., Singh, R., and Gill, B. S. (1999). Effect of pre-planting tillage on crop yields and weed biomass in a rice–wheat system on a sandy loam soil in Punjab. *Soil Till. Res.* **52,** 83–89.

Gallandt, E. R. (2006). How can we target the weed seedbank? *Weed Sci.* **54,** 588–596.

Gallandt, E. R., Liebman, M., and Huggins, D. R. (1999). Improving soil qualities: Implications for weed management. *J. Crop Prod.* **2,** 95–121.

Gangwar, K. S., Chaudhary, V. P., Gangwar, B., and Pandey, D. K. (2009). Effect of crop establishment and tillage practices in rice (*Oryza sativa*)-based cropping systems. *Indian J. Agric. Sci.* **79,** 334–339.

Gangwar, K. S., Gill, M. S., Tomar, O. K., and Pandey, D. K. (2008). Effect of crop establishment methods on growth, productivity and soil fertility of rice (*Oryza sativa*)-based cropping systems. *Indian J Agron.* **53,** 102–106.

Gangwar, K. S., Singh, K. K., and Sharma, S. K. (2004). Effects of tillage on growth, yield and nutrient uptake in wheat after rice in Indo-Gangetic Plains of India. *J. Agric. Sci.* **142,** 453–459.

Gardner-Outlaw, T., and Engelman, R. (1997). Sustaining Water, Easing Scarcity: A Second Update. Population Action International, Washington, DC, 19 pp.

Garrity, D. P., Movillon, M., and Moody, K. (1992). Differential weed suppression ability in upland rice cultivars. *Agron. J.* **84,** 586–591.

Gathala, M. K., Ladha, J. K., Kumar, V., Saharawat, Y. S., Kumar V., Sharma, P. K., Sharma, S., and Pathak, H. (2011). Tillage and crop establishment affects sustainability of South Asian rice-wheat system. *Agron. J.* (In press).

Gibson, K. D., Fischer, A. J., Foin, T. C., and Hill, J. E. (2003). Crop traits related to weed suppression in water-seeded rice (*Oryza sativa* L.). *Weed Sci.* **51,** 87–93.

GOI (Government of India) (2011). The Mahatma Gandhi National Rural Employment Guarantee Act 2005. Ministry of Rural Development, Government of India. Online available at: http://nrega.nic.in/netnrega/home.aspx (Accessed on 28 March 2011).

Golden, B. R., Slaton, N. A., Norman, R. J., Wilson, C. E. Jr., and DeLong, R. E. (2009). Evaluation of polymer-coated urea for direct-seeded, delayed-flood rice production. *Soil Sci. Soc. Am. J.* **73,** 375–383.

Gopal, R., Jat, R. K., Malik, R. K., Kumar, V., Alam, M. M., Jat, M. L., Mazid, M. A., Saharawat, Y. S., McDonald, A., and Gupta, R. (2010). Direct Dry Seeded Rice Production Technology and Weed Management in Rice Based Systems. Technical Bulletin. International Maize and Wheat Improvement Center, New Delhi, India, 28pp.

Gravois, K. A., and Helms, R. S. (1992). Path analysis of rice yield and yield components as affected by seeding rate. *Agron. J.* **84,** 1–4.

Gressel, J. (2002). Preventing, delaying and mitigating gene flow from crops—Rice as an example. *In* "Proceedings of the Seventh International Symposium on the Biosafety of Genetically Modified Organisms, Beijing, China", pp. 59–77.

Gressel, J., and Valverde, B. E. (2009). A strategy to provide long-term control of weedy rice while mitigating herbicide resistance transgene flow, and its potential use for other crops with related weeds. *Pest Manage. Sci.* **65,** 723–731.

Grigg, D. E. (1974). The Agricultural Systems of the World: An Evolutionary Approach. Cambridge University Press, Cambridge, UK.

Gunawardana, D. (2008). Harvesting. *Rice Today*, 36–39.

Gupta, R. K., Ladha, J. K., Singh, S., Singh, R., Jat, M. L., Saharawat, Y., Singh, V. P., Singh, S. S., Singh, G., Sah, G., Gathala, M., Sharma, R. K., *et al.* (2006). Production Technology for Direct Seeded Rice. Technical Bulletin Series 8. *In* "Rice–Wheat Consortium for the Indo-Gangetic Plains, New Delhi, India," 14pp.

Gupta, R. K., Naresh, R. K., Hobbs, P. R., and Ladha, J. K. (2002). Adopting conservation agriculture in rice–wheat systems of the Indo-Gangetic Plains—New opportunities for saving on water. *In* "Proceedings of the International Workshop on Water-Wise Rice Production" (B. A. M. Bouman, H. Hengsdijk, B. Hardy, B. Bindraban, T. P. Toung, and J. K. Ladha, Eds.), pp. 207–222. International Rice Research Institute, Los Baños, Philippines.

Gupta, R., and Seth, A. (2007). A review of resource conserving technologies for sustainable management of the rice–wheat cropping systems of the Indo-Gangetic Plains (IGP). *Crop Prot.* **26,** 436–447.

Guyer, R., and Quadranti, M. (1985). Effect of seed rate and nitrogen level on the yield of direct wet-seeded rice. “Proceedings of the 10th Asian-Pacific Weed Science Society Conference”, pp. 304–311. Chiangmai, Thailand.

Guzman Garcia, P., and Nieto Illidge, L. (1992). Nuevas densidades y fertilización para disminuir *Rhizoctonia solani*. *Arroz* **41,** 10–14.

Haefele, S. M., Johnson, D. E., M’bodj, D. M., Wopereis, M. C. S., and Miezan, K. M. (2004). Field screening of diverse rice genotypes for weed competitiveness in irrigated lowland ecosystems. *Field Crops Res.* **88,** 39–56.

Hafeez, M. M., Bouman, B. A. M., Van de Giesen, N., and Vlek, P. (2007). Scale effects on water use and water productivity in a rice-based irrigation system (UPRIIS) in the Philippines. *Agric. Water Manage.* **92,** 81–89.

Harada, H., Hitomi, K., and Hayato, S. (2007). Reduction in greenhouse gas emissions by no-tilling rice cultivation in Hachirogata polder, northern Japan: Life-cycle inventory analysis. *Soil Sci. Plant Nutr.* **53,** 668–677.

Harrington, L. W., and Hobbs, P. R. (2009). The Rice-Wheat Consortium and the Asian Development Bank: A history. *In* “Integrated Crop and Resource Management in the Rice-Wheat System” (J. K. Ladha, Yadvinder Singh, O. Erenstein, and B. Hardy, Eds.), pp. 3–67. International Rice Research Institute, Los Baños, Philippines.

Harris, D., Tripathi, R. S., and Joshi, A. (2002). On-farm seed priming to improve crop establishment and yield in dry direct-seeded rice. *In* “Direct Seeding: Research Strategies and Opportunities”. (S. Pandey, M. Mortimer, L. Wade, T. P. Tuong, K. Lopez, and B. Hardy, Eds.), Proceedings of the International Workshop on Direct Seeding in Asian Rice Systems: Strategic Research Issues and Opportunities, 25–28, January 2000, Bangkok, Thailand, pp. 231–240. International Rice Research Institute, Los Baños, Philippines.

Heap, I. (2010). International Survey of Herbicide Resistant Weeds, www.weedscience.com(accessed August 2010).

Heydecker, W. (1960). Can we measure seedling vigor? *Proc. Int. Seed Test. Assoc.* **25,** 498–512.

Hill, J. E., Bayer, D. E., Bocchi, S., and Clampett, W. S. (1991). Direct-seeded rice in the temperate climates of Australia, Italy, and the United States. *In* “Direct-Seeded Flooded Rice in the Tropics”, pp. 91–102. International Rice Research Institute, Los Baños, Philippines.

Hill, J. E., Roberts, S. R., Brandon, D. M., Scardaci, S. C., Williams, J. F., and Mutters, R. G. (1997). Rice Production in California. www.plantsciences.ucdavis.edu/uccerice/PRODUCT/prod.htm.

Hiraoka, H., and Ho, N. (1996). Agronomy in direct-seeding. *In* “Recent Advances in Malaysian Rice Production: Direct Seeding Culture in the Muda Area” (Y. Morooka, *et al.*, Eds.), pp. 131–157. Muda Agricultural Development Authority and Japan International Research Center for Agricultural Sciences, Ampang Jajar, Alor Setar, Malaysia.

Ho, N. K. (1996). Introducing integrated weed management in Malaysia. *In* “Herbicides in Asian Rice: Transitions in Weed Management” (R. Naylor, Ed.), pp. 167–182. Stanford University, California, International Rice Research Institute, Philippines.

Ho, N. K., and Itoh, K. (1991). Changes in the weed flora and their distribution in the Muda area. *In* “Paper Presented at the Eight MADA/TARC Quarterly Meeting,” 3 November 1991, Alor Setar, Malaysia.

Ho, N. K., and Romli, Z. (2002). Impact of direct seeding on rice cultivation: Lessons from the Muda area of Malaysia. *In* “Direct Seeding: Research Strategies and Opportunities”

(S. Pandey, M. Mortimer, L. Wade, T. P. Tuong, K. Lopez, and B. Hardy, Eds.), pp. 87–98. International Rice Research Institute, Los Baños, Philippines.

Hobbs, P. R., and Gupta, R. K. (2000). Sustainable resource management in intensively cultivated irrigated rice–wheat cropping systems of the Indo-Gangetic Plains of South Asia: Strategies and options. *In* "International Conference on Managing Natural Resources for Sustainable Production in 21st Century", 14–18 February 2000, New Delhi, India, pp. 584–592.

Hobbs, P. R., and Gupta, R. K. (2003). Resource-conserving technologies for wheat in the rice–wheat system. *In* "Improving the Productivity and Sustainability of Rice–Wheat Systems: Issues and Impacts" (J. K. Ladha, J. E. Hill, J. M. Duxbury, R. K. Gupta, and R. J. Buresh, Eds.), pp. 149–172, ASA Special Publication Number 65. ASA-CSSA-SSSA, Madison, Wisconsin, USA.

Hobbs, P. R., Singh, Y., Giri, G. S., Lauren, J. G., and Duxbury, J. M. (2002). Direct seeding and reduced tillage options in the rice-wheat systems of the Indo-Gangetic Plains of South Asia. *In* "Direct seeding in Asian Rice Systems: Strategic Research Issues and Opportunities" (S. Pandey, M. Mortimer, L. Wade, T. P. Tuong, and B. Hardy, Eds.), pp. 201–205. International Rice Research Institute, Los Baños, Philippines.

Horie, T. (2001). Increasing yield potential in irrigated rice: Breaking the barrier. *In* "Rice Research for Food Security and Poverty Alleviation". (S. Peng and B. Hardy, Eds.), Proc. Int. Rice Res. Conf., IRRI, Los Baños, Philippines, pp. 3–25.

Horie, T., Shiraiwa, T., Homma, K., Katsura, K., Maeda, Y., and Yoshida, H. (2004). Can yields of lowland rice resume the increases that they showed in the 1980's. *In* "Proceedings of the 4th International Crop Science Congress, New Directions for a Diverse Planet, 26 September–1 October, 2004, Brisbane, Australia," www.cropscience.org.au.

Hou, A. X., Chen, G. X., Wang, Z. P., Van Cleemput, O., and Patrick, W. H. Jr. (2000). Methane and nitrous oxide emissions form a rice field in relation to soil redox and microbiological processes. *Soil Sci. Soc. Am. J.* **64,** 2180–2186.

Houghton, J. T., Meira Filho, L. G., Callander, B. A., Harris, N., Katterberg, A., and Maskell, K. (1996). IPCC report on climate change: The science of climate change. "WG1 Contribution to the IPCC Second Assessment Report on Methane Emission from Rice Cultivation", Cambridge University Press, Cambridge, UK.

Huan, T. T. N., Tan, P. S., and Hiraoka, H. (1999). Path-coefficient analysis of direct-seeded rice yield and yield components as affected by seeding rates. *Omonrice* **7,** 104–111.

Hukkeri, S. B., and Sharma, A. K. (1980). Water use efficiency of transplanted and direct-sown rice after different water management practices. *Indian J. Agric. Sci.* **50,** 240–243.

Humphreys, E., Kukal, S. S., Christen, E. W., Hira, G. S., Balwinder-Singh, Sudhir-Yadav, and Sharma, R. K. (2010). Halting the groundwater decline in north-west India—Which crop technologies will be winners? *Adv. Agron.* **109,** 155–217.

Humphreys, E., Meisner, C., Gupta, R., Timsina, J., Beecher, H. G., Tang, Y. L., Singh, Y., Gill, M. A., Masih, I., Zheng, J. G., and Thompson, J. A. (2005). Water savings in rice-wheat systems. *Plant Prod. Sci.* **8,** 242–258.

IPCC-Intergovernmental Panel on Climate Change (2007). Climate change 2007: The physical science basis.(S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K. Averyt, M. Tignor, and H. L. Milier, Eds.), *In* "Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change". Cambridge University Press, Cambridge, United Kingdom, New York, NY, USA.

IRRI (1984). Annual Report for 1983. International Rice Research Institute, Los Baños, Philippines.

IRRI (1994). Program Report for 1993. International Rice Research Institute, Los Baños, Philippines.

IRRI (2008). Background Paper: The Rice Crisis: What Needs to Be Done? IRRI, Los Baños, Philippines, www.irri.org/12pp.

IRRI (2010). Site-specific nutrient management, www.irri.org/irrc/ssnm. Accessed 30 April 2010.

Ishaq, M., Hassan, A., Saeed, M., Ibrahim, M., and Lal, R. (2001). Subsoil compaction effects on crops in Punjab. Pakistan. I. Soil physical properties and crop yield. *Soil Till. Res.* **59,** 57–65.

Ishibashi, E., Akai, N., Ohya, M., Ishii, T., and Tsuruta, H. (2001). The influence of no-tilled direct-seeding cultivation on methane emission from three rice paddy fields in Okayama, Western Japan. 2. The relationship between the continuation of no-tilled cultivation and methane emission. *Jpn. J. Soil Sci. Plant Nutr.* **72,** 542–549.

Ishibashi, E., Yamamoto, S., Akai, N., and Tsuruta, H. (2007). The influence of no-tilled direct seeding cultivation on greenhouse gas emissions from rice paddy fields in Okayama, Western Japan. 4. Major factors controlling nitrous oxide emission from rice paddy fields under no-till direct seeding cultivation. *Jpn. J. Soil Sci. Plant Nutr.* **78,** 453–463.

Ishibashi, E., Yamamoto, S., Akai, N., and Tsuruta, H. (2009). The influence of no-tilled direct seeding cultivation on greenhouse gas emissions from rice paddy fields in Okayama, Western Japan. 5. Annual emission of CH_4, N_2O and CO_2 from rice paddy fields under different cultivation methods and carbon sequestration into paddy soils. *Jpn. J. Soil Sci. Plant Nutr.* **80,** 123–135.

Islam, M. F., Sarkar, M. A. R., Islam, M. S., Parveen, S., and Hossain, M. S. (2008). Effects of crop establishment methods on root and shoot growth, lodging behavior of Aus rice. *Int. J. Biol. Res.* **5,** 60–64, (Paper No. 71).

Ismail, A. M., Ella, E. S., Vergara, G. V., and Mackill, D. J. (2009). Mechanisms associated with tolerance to flooding during germination and early seedling growth in rice (*Oryza sativa*). *Ann. Bot.* **103,** 197–209.

Isvilanonda, S. (2002). Development trends and farmers' benefits in the adoption of wet-seeded rice in Thailand. *In* "Direct Seeding: Research Strategies and Opportunities. Proceedings of the International Workshop on Direct Seeding in Asian Rice Systems: Strategic Research Issues and Opportunities" (S. Pandey, M. Mortimer, L. Wade, T. P. Tuong, K. Lopez, and B. Hardy, Eds.), pp. 115–124. International Rice Research Institute, Los Baños, Philippines, 25–28 January 2000, Bangkok, Thailand.

IWMI (2000). World Water Supply and Demand 1995 to 2025. Draft Report Prepared for World Water Vision International Water Management Institute, Colombo, Sri Lanka, www.iwmi.cgiar.org/pubs/WWVisn/WWSDOpen.htm.

Jaafar, M. H., Amit, D. R., and Tulas, M. T. (1995). Wet-seeded rice in Sabah (Malaysia). *In* "Constraints, Opportunities and Innovations for Wet-Seeded Rice" (K. Moody, Ed.), IRRI Discussion Paper Series No. 10, pp. 63–72. International Rice Research Institute, Los Baños, Philippines.

Jacob Spafford, H., Minkey, D. M., Gallagher, R. S., and Borger, C. P. (2006). Variation in postdispersal weed seed predation in a crop field. *Weed Sci.* **54,** 148–155.

Jalota, S. K., and Arora, V. K. (2002). Model-based assessment of water balance components under different cropping systems in north-west India. *Agric. Water Manage.* **57,** 75–87.

Jannink, J. L., Orf, J. H., Jordan, N. R., and Shaw, R. G. (2000). Index selection for weed-suppressive ability in soybean. *Crop Sci.* **40,** 1087–1094.

Jat, M. L., Chandna, P., Gupta, R., Sharma, S. K., and Gill, M. A. (2006). Laser land leveling: A precursor technology for resource conservation. *In* "Rice-Wheat Consortium Technical Bulletin 7. Rice-Wheat Consortium for the Indo-Gangetic Plains, New Delhi, India," 48 p.

Jat, M. L., Gathala, M. K., Ladha, J. K., Saharawat, Y. S., Jat, A. S., Vipin, Kumar, Sharma, S. K., Kumar, V., and Gupta, R. K. (2009). Evaluation of precision land leveling

and double zero-till systems in the rice-wheat rotation: Water use, productivity, profitability and soil physical properties. *Soil Till. Res.* **105,** 112–121.

Jennings, P. R., and Jesus, J. D. Jr. (1968). Studies on competition in rice. I. Competition in mixtures of varieties. *Evolution* **22,** 119–124.

Jiang, Y. (2009). China's water scarcity. *J. Environ. Manage.* **90,** 3185–3196.

Johnson, D. E., and Mortimer, A. M. (2005). Issues for integrated weed management and decision support in direct-seeded rice. *In* "Rice Is Life: Scientific Perspectives for the 21st Century" (K. Toriyama, K. L. Heong, and B. Hardy, Eds.), pp. 211–214. International Rice Research Institute, Los Baños, Philippines and Japan International Research Center for Agricultural Sciences, Tsukuba, Japan.

Johnson, D. E., White, J. L., and Mortimer, M. (2003). Development of sustainable weed management systems in direct seeded, irrigated rice Final technical report, the United Kingdom Department for International Development (DFID): Crop Protection Program-Project-R7377/ZA0299. Natural Resources Institute, University of Greenwich, Chatham, Kent. 55pp.

Jones, D. B., and Snyder, G. H. (1987). Seeding rate and row spacing effects on yield and yield components of drill-seeded rice. *Agron. J.* **79,** 623–626.

Jones, M. P., Dingkuhn, M., Aluko, G. K., and Semon, M. (1997b). Interspecific *Oryza sativa* L. x *O. glaberrima* Steud. progenies in upland rice improvement. *Euphytica* **92,** 237–246.

Jones, M. P., Mande, S., and Aluko, K. (1997a). Diversity and potential of *Oryza glaberrima* (Steud.) in upland rice breeding. *Breed. Sci.* **47,** 395–398.

Jordan, N. (1993). Prospects for weed control through interference. *Ecol. Appl.* **3,** 84–91.

Jugsujinda, A., Delaune, R. D., and Lindau, C. W. (1996). Factors controlling carbon dioxide and methane production in acid sulfate soils. *Water Air Soil Pollut.* **87,** 345–355.

Kabir, M. H., Saha, A., Mollah, I. U., Kabir, M. S., and Rahman, F. (2008). Effect of crop establishment methods and weed management practices on the productivity of boro rice in lowland ecosystem. *Int. J. Biol. Res.* **5,** 42–51.

Kahlown, M. A., Raoof, A., Zubair, M., and Kemper, W. D. (2007). Water use efficiency and economic feasibility of growing rice and wheat with sprinkler irrigation in the Indus Basin of Pakistan. *Agric. Water Manage.* **87,** 292–298.

Karim, R. S. M., Man, A. B., and Sahid, I. B. (2004). Weed problems and their management in rice fields of Malaysia: An overview. *Weed Biol. Manage.* **4,** 177–186.

Kato, Y., and Katsura, K. (2010). Panicle architecture and grain number in irrigated rice, grown under different water management regimes. *Field Crops Res.* **117,** 237–244.

Kato, Y., and Okami, M. (2010). Root growth dynamics and stomatal behaviour of rice (*Oryza sativa* L.) grown under aerobic and flooded conditions. *Field Crops Res.* **117,** 9–17.

Kato, Y., Okami, M., and Katsura, K. (2009). Yield potential and water use efficiency of aerobic rice (*Oryza sativa* L.) in Japan. *Field Crops Res.* **113,** 328–334.

Katsura, K., Okami, M., Mizunuma, H., and Kato, Y. (2010). Radiation use. N accumulation and biomass production of high-yielding rice in aerobic culture. *Field Crops Res.* **117,** 81–89.

Kawano, K., Gonzalez, H., and Lucena, M. (1974). Intraspecific competition with weeds, and spacing response in rice. *Crop Sci.* **14,** 841–845.

Kawasaki, K. (1989). Paddy field size and mechanised operations. *Trans. Jpn. Soc. Irrig. Drain. Reclam. Eng.* **57,** 29–34.

Khan, M. A. H., Alam, M. M., Hossain, M. I., Rashid, M. H., Mollah, M. I. U., Quddus, M. A., Miah, M. I. B., Sikder, M. A. A., and Ladha, J. K. (2009). Validation and delivery of improved technologies in the rice-wheat ecosystem in Bangladesh. *In* "Integrated Crop and Resource Management in the Rice–Wheat System of South Asia" (J. K. Ladha, Y. Singh, O. Erenstein, and B. Hardy, Eds.), pp. 197–220. International Rice Research Institute, Los Baños, Philippines.

Khush, G. S. (2004). Harnessing science and technology for sustainable rice-based production systems. Proceedings of FAO Rice Conference "Rice is life". *Int. Rice Comm. Newsl.* **53,** 17–23.

Kim, S. C., and Ha, W. G. (2005). Direct-seeding and weed management in Korea. *In* "Rice Is Life: Scientific Perspectives for the 21st Century" (K. Toriyama, K. L. Heong, and B. Hardy, Eds.), pp. 181–184. International Rice Research Institute, Los Baños, Philippines and Japan International Research Center for Agricultural Sciences, Tsukuba, Japan.

Kim, S. C., Oh, Y. J., and Kwon, Y. W. (1992). Weed flora of agricultural area in Korea. *Korean J. Weed Sci.* **12,** 317–334.

Kimura, S., Imazono, S., and Yaji, Y. (1999). Development of labor saving operation technique by making large scale paddy field and direct seeding cultivation of rice in Tohoku District. Part 2. Development of technique for automatic precision laser-levelling system. *J. Jpn. Farm Work Res.* **34,** 113–121.

Kirchhof, G., and So, H. B. (1996). The effect of puddling intensity and compaction on soil properties, rice and mungbean growth: A mini-ricebed study. *In* "Proceedings of the ACIAR International Workshop on Management of Clay Soils for Rainfed Lowland Rice-based Cropping Systems, November 24–25, Manila, Philippines," pp. 51–70.

Ko, J. Y., and Kang, H. W. (2000). The effects of cultural practices on methane emission from rice fields. *Nutr. Cycl. Agroecosyst.* **58,** 311–314.

Ko, J. Y., Lee, J. S., Kim, M. T., Kang, H. W., Kang, U. G., Lee, D. C., Shin, Y. G., Kim, K. Y., and Lee, K. B. (2002). Effects of cultural practices on methane emission in tillage and no-tillage practice from rice paddy fields. *Korean J. Soil Sci. Fert.* **35,** 216–222.

Krausz, J. P., and Groth, D. E. (2008). Seedling disease control. *In* "Texas Rice Production Guidelines" (M. O. Way and J. Cockrell, Eds.), p. 14. Agri Life Ext. Ser. B-6131, 2–08.

Kukal, S. S., and Aggarwal, G. C. (2003). Puddling depth and intensity effects in rice-wheat system on a sandy loam soil. I. Development of subsurface compaction. *Soil Till. Res.* **72,** 1–8.

Kumar, V., Bellinder, R. R., Brainard, D. C., Malik, R. K., and Gupta, R. K. (2008b). Risks of herbicide-resistant rice in India: A review. *Crop Prot.* **27,** 320–329.

Kumar, V., Bellinder, R. R., Gupta, R. K., Malik, R. K., and Brainard, D. C. (2008a). Role of herbicide-resistant rice in promoting resource conservation technologies in rice–wheat cropping systems of India: A review. *Crop Prot.* **27,** 290–301.

Kumar, V., Ladha, J. K., and Gathala, M. K. (2009). Direct drill-seeded rice: A need of the day. *In* "Annual Meeting of Agronomy Society of America, Pittsburgh, November 1–5, 2009," http://a-c-s.confex.com/crops/2009am/webprogram/Paper53386.html.

Ladha, J. K., Dawe, D., Pathak, H., Padre, A. T., Yadav, R. L., Singh, B., Singh, Y., Singh, Y., Singh, P., Kundu, A. L., Sakal, R., Ram, N., *et al.* (2003). How extensive are yield declines in long-term rice–wheat experiments in Asia? *Field Crops Res.* **81,** 159–180.

Ladha, J. K., Kumar, V., Alam, M. M., Sharma, S., Gathala, M., Chandna, P., Saharawat, Y. S., and Balasubramanian, V. (2009). Integrating crop and resource management technologies for enhanced productivity, profitability, and sustainability of the rice-wheat system in South Asia. *In* "Integrated Crop and Resource Management in the Rice–Wheat System of South Asia" (J. K. Ladha, Y. Singh, O. Erenstein, and B. Hardy, Eds.), pp. 69–108. International Rice Research Institute, Los Baños, Philippines.

Ladha, J. K., Pathak, H., Tirol-Padre, A., Dawe, D., and Gupta, R. K. (2003b). Productivity trends in intensive rice-wheat cropping systems in Asia. *In* "Improving the Productivity and Sustainability of Rice-Wheat Systems: Issues and Impacts" (J. K. Ladha, J. E. Hill, J. M. Duxbury, R. K. Gupta, and R. J. Buresh, Eds.), pp. 45–76. ASA, CSSA, SSSA, Madison, WI, ASA Special Publication 65.

Lafitte, H. R., Courtois, B., and Arradeau, M. (2002). Genetic improvement of rice in aerobic systems: Progress from yield to genes. *Field Crops Res.* **75,** 171–190.

Lantican, M. A., Lampayan, R. M., Bhuiyan, S. I., and Yadav, M. K. (1999). Determinants of improving productivity of dry-seeded rice in rainfed lowlands. *Exp. Agric.* **35,** 127–140.

Lee, M. H., Kim, J. K., Kim, S. S., and Park, S. T. (2002). Status of dry-seeding technologies for rice in Korea. In "Direct Seeding: Research Strategies and Opportunities" (S. Pandey, M. Mortimer, L. Wade, T. P. Tuong, K. Lopez, and B. Hardy, Eds.), pp. 161-176. International Rice Research Institute, Los Baños, Philippines.

Liebman, M., and Gallandt, E. (1997). Many little hammers: Ecological approaches for management of crop-weed interactions. *In* "Ecology in Agriculture (Physiological Ecology)" (L. E. Jackson, Ed.), pp. 291–346. Academic Press, San Diego, CA.

Liu, C. M., and Xia, J. (2004). Water problems and hydrological research in the Yellow River and the Huai and Hai River Basins of China. *Hydrol. Proc.* **18,** 2197–2210.

Liu, C. M., and Yu, J. J. (2001). Groundwater exploitation and its impact on the environment in the North China Plain. *Water Int.* **26,** 265–272.

Lotz, L. A. P., Wallinga, J., and Kropff, M. J. (1995). Crop-weed interactions: Quantification and prediction. *In* "Ecology and Integrated Farming Systems" (D. M. Glen, M. P. Greaves, and H. M. Anderson, Eds.), pp. 31–47. Wiley and Sons, Chichester, U. K.

Mackill, D. J., Coffman, W. R., and Garrity, D. P. (1996). Rainfed Lowland Rice Improvement. International Rice Research Institute, Manila, Philippines, 242 p.

Mahata, K. R., Sen, H. S., Pradhan, S. K., and Mandal, L. N. (1990). No till and dry ploughing compared with puddling for wet season rice on alluvial sandy clay loam in eastern India. *J. Agric. Sci.* **114,** 79–86.

Mai, V., Chien, H. V., Van, A. V., Thi, V., Suong, T., and Thiet, L. V. (2000). Rice seed contamination in Vietnam. *In* "Proceedings of the International Symposium on Wild and Weedy Rices in Rice Ecosystems in Asia: A Review" (B. B. Baki, D. V. Chin, and M. Mortimer, Eds.), pp. 17–19. International Rice Research Institute, Los Baños, Philippines.

Malik, R. K., Yadav, A., and Singh, S. (2005). Resource conservation technologies in rice–wheat cropping system of Indo-Gangetic Plain. *In* "Conservation Agriculture: Status and Prospects" (L. P. Abrol, R. K. Gupta, and R. K. Malik, Eds.), pp. 13–22. Centre for Advancement of Sustainable Agriculture, New Delhi, India.

Maneechote, C., Jamjod, S., and Rerkasem, B. (2004). Invasion of weedy rice in rice fields in Thailand: Problems and management. *Int. Rice Res. Notes* **29,** 20–22.

Masscheleyn, P. H., Delaune, R. D., and Patrick, W. H. Jr. (1993). Methane and nitrous oxide emission from laboratory measurements of rice soil suspension: Effect of soil oxidation-reduction status. *Chemosphere* **26,** 251–260.

Matsushima, S. (1957). Analysis of developmental factors determining yield and yield prediction in lowland rice. *Bull. Natl. Inst. Agric. Sci. Ser.* **A5,** 1–271.

McDonald, A. J., Riha, S. J., Duxbury, J. M., and Lauren, J. G. (2006). Wheat responses to novel rice cultural practices and soil moisture conditions in the rice–wheat rotation of Nepal. *Field Crops Res.* **98,** 116–126.

McKenzie, K. S., Johnson, C. W., Tseng, S. T., Oster, J. J., and Brandon, D. M. (1994). Breeding improved rice cultivars for temperate regions: A case study. *Aust. J. Exp. Agric.* **34,** 897–905.

Meelu, O. P., Beri, V., Sharma, K. N., Jalota, S. K., and Sandhu, B. S. (1979). Influence of paddy and corn in different rotations on wheat yield, nutrient removal and soil properties. *Plant Soil* **51,** 51–571.

Meisner, C. A., Amin, M. R., Duxbury, J. M., Lauren, J. G., Badaruddin, M., and Ahmed, M. (2002). Nitrogen and irrigation management for direct-seeded rice on light soils in a rice-wheat cropping system. *In* "Proceedings 17th World Congress of Soil Sciences (WCSS), 14–21 August 2002, Bangkok, Thailand," pp. 780-1–780-6.

Mispan, M., and Baki, B. B. (2008). They stand among equals: Descriptive analysis on the new biotypes of weedy rice (*Oryza sativa* L.) in Malaysia. *In* "Abstracts V International Weed Science Congress," Vancouver, Canada, Abstract 724.

Mitchell, J., Fukai, S., and Basnayake, J. (2004). Grain yield of direct seeded and transplanted rice in rainfed lowlands of South East Asia. *In* "Proceedings of 4th International Crop Science Congress," 26 September–October 2004, Brisbane, Queensland, Australia (on CD).

Mithrasena, Y. J. P. K., and Adikari, W. P. (1986). Effect of density on sheath blight (ShB) incidence. *Int. Rice Res. Notes* **11,** 20–21.

Mohler, C. L. (1996). Ecological bases for the cultural control of annual weeds. *J. Prod. Agric.* **9,** 468–474.

Mohler, C. L., and Callaway, M. B. (1991). Effects of tillage and mulch on emergence and survival of weeds in sweet corn. *J. Appl. Ecol.* **29,** 21–34.

Mohler, C. L., and Galford, A. E. (1997). Weed seedling emergence and seed survival: Separating the effects of seed position and soil modification by tillage. *Weed Res.* **37,** 147–155.

Mohler, C. L., and Teasdale, J. R. (1993). Response of weed emergence to rate of *Vicia villosa* Roth and *Secale cereale* L. residue. *Weed Res.* **33,** 487–499.

Molden, D. (1997). Accounting for Water Use and Productivity. SWIM Paper No. 1 International Irrigation Management Institute, Colombo, Sri Lanka.

Molden, D., Frenken, K., Barker, R., de Fraiture, C., Mati, B., Svendsen, M., Sadoff, C., and Finlayson, C. M. (2007). Water for Food, Water for Life: A Comprehensive Assessment of Water Management in Agriculture. Earthscan/International Water Management Institute, London/Colombo, Sri Lanka.

Moody, K. (1977). Weed control in multiple cropping. "Cropping Systems Research and Development for the Asian Rice Farmer", pp. 281–293. International Rice Research Institute, Los Baños, Laguna, Philippines.

Morita, S., and Yamazaki, K. (1993). Root system. *In* "Science of the Rice Plant" (T. Matsuo and K. Hoshikawa, Eds.), Morphology, vol. 1, pp. 161–186. Food and Agriculture Policy Research Center, Tokyo.

Mortimer, A. M., and Hill, J. E. (1999). Weed species shifts in response to broad spectrum herbicides in sub-tropical and tropical crops. *Brighton Crop Prot. Conf.* **2,** 425–437.

Mortimer, A. M., Riches, C. R., Mazid, M., Pandey, S., and Johnson, D. E. (2008). Issues related to direct seeding of rice in rainfed cropping systems in northwest Bangladesh. *In* "Direct seeding of rice and weed management in the irrigated rice-wheat cropping system of the Indo-Gangetic Plains" (Y. Singh, V. P. Singh, B. Chauhan, A. Orr, A. M. Mortimer, D. E. Johnson, and B. Hardy, Eds.), International Rice Research Institute, Los Baños, Philippines, and Directorate of Experiment Station, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttaranchal, India, 272 p.

Musa, M. M. (1969). Some aspects of nitrogen transformation in Gezira soil. *In* "Cotton Growth in Gezira Environment" (M. A. Siddiq and L. C. Hughes, Eds.), pp. 29–38.

MWR. (2007). The 11th Five-Year Plan of National Water Resources Development, Gazette of the Ministry of Water Resources of the P.R. China 2007. pp. 34–48.

My, T. V., Tuong, T. P., Xuan, V. T., and Nghiep, N. T. (1995). Dry seeding rice for increased cropping intensity in Long An Province, Vietnam. "Vietnam and IRRI, a Partnership in Rice Research", pp. 111–122. Ministry of Agriculture and Food Industry, Vietnam and International Rice Research Institute, Los Baños, Philippines.

Ni, H., Moody, K., Robles, R. P., Paller, E. C., and Lales, J. S. (2000). *Oryza sativa* plant traits conferring competitive ability against weeds. *Weed Sci.* **48,** 200–204.

Noldin, J. A., Chandler, J. M., Ketchersid, M. L., and McCauley, G. N. (1999a). Red rice (*Oryza sativa*) biology. II. Ecotype sensitivity to herbicides. *Weed Technol.* **13,** 19–24.

Noldin, J. A., Chandler, J. M., and McCauley, G. N. (1999b). Red rice (*Oryza sativa*) biology. I. Characterization of red rice ecotypes. *Weed Technol.* **13,** 12–18.
OECD. (2000). Environmental Indicators for Agriculture Methods and Results. Executive summary. OECD, Paris.
Ota, Y., and Nakayama, M. (1970). Effect of seed coating with calcium peroxide on germination under submerged condition on rice plant. *Proc. Crop Sci. Soc. Jpn.* **39,** 535–536 (in Japanese with English summary).
Ottis, B. V., Smith, K. L., Scott, R. C., and Talbert, R. E. (2005). Rice yield and quality as affected by cultivar and red rice (*Oryza sativa*) density. *Weed Sci.* **53,** 499–504.
Oussible, M., Crookstan, R. K., and Larson, W. E. (1992). Subsurface compaction reduces the root and shoot growth and grain yield of wheat. *Agron. J.* **84,** 34–38.
Pal, S., Datta, S. P., Rattan, R. K., and Singh, A. K. (2008). Diagnosis and amelioration of iron deficiency under aerobic rice. *J. Plant Nutr.* **31,** 919–940.
Pandey, S., and Velasco, L. (2002). Economics of direct seeding in Asia: Patterns of adoption and research priorities. *In* "Direct Seeding: Research Strategies and Opportunities" (S. Pandey, M. Mortimer, L. Wade, T. P. Tuong, K. Lopez, and B. Hardy, Eds.), pp. 3–14. International Rice Research Institute, Los Baños, Philippines.
Pandey, S., and Velasco, L. (2005). Trends in crop establishment methods in Asia and research issues. *In* "Rice Is Life: Scientific Perspectives for the 21st Century" (K. Toriyama, K. L. Heong, and B. Hardy, Eds.), pp. 178–181. International Rice Research Institute, Los Baños, Philippines and Japan International Research Center for Agricultural Sciences, Tsukuba, Japan.
Pandey, S., and Velasco, L. E. (1998). Economics of direct-seeded rice in Iloilo: Lessons from nearly two decades of adoption. Social Sciences Division Discussion Paper International Rice Research Institute, Manila, Philippines.
Pandey, S., Velasco, L. E., and Suphanchalmat, N. (2002). Economics of direct seeding in northern Thailand. *In* "Direct Seeding: Research Strategies and Opportunities" (S. Pandey, M. Mortimer, L. Wade, T. P. Tuong, K. Lopez, and B. Hardy, Eds.), pp. 139–150. International Rice Research Institute, Los Baños, Philippines.
Pandey, S., Velasco, L., Masicat, P., and Gagalac, F. (1995). An economic analysis of rice establishment methods in Pangasinan, Central Luzon. Social Sciences Division Discussion Paper International Rice Research Institute, Los Baños, Philippines.
Pantone, D. J., and Baker, J. B. (1991). Weed-crop competition models and response-surface analysis of red rice competition in cultivated rice: A review. *Crop Sci.* **31,** 1105–1110.
Parihar, S. S. (2004). Effect of crop-establishment method, tillage, irrigation and nitrogen on production potential of rice-wheat cropping system. *Indian J. Agron.* **49,** 1–5.
Park, S. T., Son, Y., Lee, S. K., and Chung, K. S. (1990). Studies on major cultivation methods for dry direct seeding. *Res. Rep. RDA (Rice)* **32,** 18–28.
Pathak, H., Saharawat, Y. S., Gathala, M. K., Mohanty, S., and Ladha, J. K. (2009). Simulating environmental impact of resource-conserving technologies in the rice-wheat system of the Indo-Gangetic Plains. *In* "Integrated Crop and Resource Management in the Rice-Wheat System of South Asia" (J. K. Ladha, Y. Singh, O. Erenstein, and B. Hardy, Eds.), pp. 321–333. International Rice Research Institute, Los Baños, Philippines.
Pathinayake, B. D., Nugaliyadde, L., and Sandanayake, C. A. (1991). Direct seeding practices for rice in Sri Lanka. *In* "Direct-Seeded Flooded Rice in the Tropics", pp. 77–90. International Rice Research Institute, Manila, Philippines.
Patrick, W. H. Jr., and Wyatt, R. (1964). Soil nitrogen loss as a result of alternate submergence and drying. *Soil Sci. Soc. Am. J.* **28,** 647–653.
Pérez de Vida, F. B., Laca, E. A., Mackill, D. J., Fernández, G. M., and Fischer, A. J. (2006). Relating rice traits to weed competitiveness and yield: A path analysis. *Weed Sci.* **54,** 1122–1131.

PhilRice (The Philippine Rice Research Institute). (2002). Integrated nutrient management for rice production. *In* "Rice Technology Bulletin No. 45" p. 24. The Philippine Rice Research Institute (PhilRice), Maligaya, Nueva Ecija, Philippines.

Ponnamperuma, F. N. (1972). The chemistry of submerged soils. *Adv. Agron.* **24,** 29–96.

Postel, S. (1997). Last Oasis: Facing Water Scarcity. Norton and Company, New York. 239 p.

Prasad, R. (2005). Rice–wheat cropping systems. *Adv. Agron.* **86,** 255–339.

Quintero, S. H. (1986). Competitive ability of different upland rice cultivars against weeds. M.S. thesis. University of the Philippines, Los Baños, College, Laguna, Philippines.

Qureshi, A. S., McCornick, P. G., Qadir, M., and Aslam, Z. (2008). Managing salinity and waterlogging in the Indus Basin of Pakistan. *Agric. Water Manage.* **95,** 1–10.

Rahman, M. B., Hossain, S. M. A., Biswas, J. C., Islam, S. A., Sarkar, A. B. S., and Rahman, M. A. (2008). Studies on the performances of wet-seeded and transplanted Aman and Boro rice. *Eco-Friendly Agric. J.* **1,** 18–25.

Ram, M., Hari, O., Dhiman, S. D., and Nandal, D. P. (2006). Productivity and economics of rice (*Oryza sativa*)—Wheat (*Triticum aestivum*) cropping system as affected by establishment methods and tillage practices. *Indian J. Agron.* **51,** 77–80.

Ramasamy, S., Susheela, C., and Sathyammorthy, K. (2006). Direct-planting system: Energy saving high output rice establishment technique for lowland. *In* "Proceedings of 26th International Rice Research Conference", p. 494.

Rao, A. N., Johnson, D. E., Sivaprasad, B., Ladha, J. K., and Mortimer, A. M. (2007). Weed management in direct-seeded rice. *Adv. Agron.* **93,** 153–255.

Rao, A. N., and Nagamani, A. (2007). Available technologies and future research challenges for managing weeds in dry-seeded rice in India. *In* "Proceedings of the 21st Asian Pacific Weed Science Society Conference from 2 to 6 October 2007, Colombo, Sri Lanka", pp. 391–491.

Rashid, M. H., Alam, M. M., Khan, M. A. H., and Ladha, J. K. (2009). Productivity and resource use of direct-(drum)-seeded and transplanted rice in puddled soils in rice-rice and rice-wheat ecosystem. *Field Crops Res.* **113,** 274–281.

Rath, B. S., Misra, R. D., Pandey, D. S., and Singh, V. P. (2000). Effect of sowing methods on growth, productivity and nutrient uptake of wheat at varying level of puddling in rice. *Indian J. Agron.* **45,** 463–469.

Redoña, E. D., and Mackill, D. J. (1996). Mapping quantitative trait loci for seedling vigor in rice using RFLPs. *Theor. Appl. Genet.* **92,** 395–402.

Reiner, W., and Milkha, S. A. (2000). The role of rice plants in regulating mechanisms of methane emissions. *Biol. Fertil. Soils* **31,** 20–29.

Renu, S., Thomas, C. G., and Abraham, C. T. (2000). Stale seedbed technique for the management of *Sacciolepis interrupta* in semi-dry rice. *Indian J. Weed Sci.* **32,** 140–145.

Rickman, J. F. (2002). Manual for Laser Land Leveling. Rice–Wheat Consortium Technical Bulletin Series 5. *In* "Rice–Wheat Consortium for the Indo-Gangetic Plains," New Delhi, India, 24 p.

Rickman, J. F., Pyseth, M., Bunna, S., and Sinath, P. (2001). Direct seeding of rice in Cambodia. *In* "Proceedings of an International Workshop", 30 October–2 November. ACIAR Proceedings No. 101, Vientiane, Laos.

Rijsberman, F. R. (2006). Water scarcity: Fact or fiction? *Agric. Water Manage.* **80,** 5–22.

Rodell, M., Velicogna, I., and Famiglietti, J. S. (2009). Satellite-based estimates of groundwater depletion in India. *Nature* **460,** 999–1002.

RWC-CIMMYT (2003). Addressing resource conservation issues in rice–wheat systems of South Asia: A resource book. *In* "Rice–Wheat Consortium for the Indo-Gangetic Plains—International Maize and Wheat Improvement Center", New Delhi, India.

RWC-IRRI. (2009). Agenda Notes. *In* "15th Regional Technical Coordination Committee Meeting", 2–3 February 2009, New Delhi, India (RWC-IRRI, New Delhi, India).

Sadras, V. O., and Calvino, P. A. (2001). Quantification of grain yield response to soil depth in soybean, maize, sunflower, and wheat. *Agron. J.* **93,** 577–583.
Saharawat, Y. S., Singh, Bhagat, Malik, R. K., Ladha, J. K., Gathala, M., Jat, M. L., and Kumar, V. (2010). Evaluation of alternative tillage and crop establishment methods in a rice-wheat rotation in North Western IGP. *Field Crops Res.* **116,** 260–267.
Saharawat, Y. S., Gathala, M., Ladha, J. K., Malik, R. K., Singh, S., Jat, M. L., Gupta, R. K., Pathak, H., and Singh, K. (2009). Evaluation and promotion of integrated crop and resource management in the rice-wheat system in northwest India. *In* "Integrated Crop and Resource Management in the Rice-Wheat System of South Asia" (J. K. Ladha, Y. Singh, O. Erenstein, and B. Hardy, Eds.), pp. 133–150. International Rice Research Institute, Los Baños, Philippines.
Saichuk, J. (2009). Louisiana Rice Production Handbook 2009. Pub. 2321 (3M) 6/09 Rev.
Saigusa, M. (2005). New fertilizer management to maximize yield and minimize environmental effects in rice culture. "Rice Is Life: Scientific Perspectives for the 21st Century. Proceedings of the World Rice Research Conference, 4–7 November 2004, Tsukuba, Japan", pp. 372–373.
Sanchez, P. A. (1973). Puddling tropical soils. 2. Effects on water losses. *Soil Sci.* **115,** 303–308.
Sangakkara, U. R., Nissanka, S. P., Marambe, B., Hurle, K., and Rubin, B. (2004). Weeds, herbicide use and resistance in rice fields of Sri Lanka. *In* "Proceedings of the 4th International Crop Science Congress: New directions for a diverse planet," Brisbane, Australia, www.cropscience.org.au/icsc2004/poster/2/4/1/378_sangakkaraur.htm#TopOfPage.
San-oh, Y., Mano, Y., Ookawa, T., and Hirasawa, T. (2004). Comparison of dry matter production and associated characteristics between direct-sown and transplanted rice plants in a submerged paddy field and relationships to planting pattern. *Field Crops Res.* **87,** 43–58.
Santhi, P. K., Ponnuswamy, K., and Kempuchetty, N. (1998). A labour saving technique in direct-sown and transplanted rice. *Int. Rice Res. Notes* **23,** 35–36.
SAS Institute (2001). SAS/STAT User's Guide Version 8.1. SAS Institute, Cary, NC, 1030 p.
Sasahara, T., Ikarashi, H., and Kambayashi, M. (1986). Genetic variations in embryo and endosperm weights, seedling growth parameters and α-amylase activity of the germinated grains in rice (*Oryza sativa* L.). *Jpn. J. Breed.* **36,** 248–261.
Sen, A., and Sharma, S. N. (2002). Zero tillage: Rice–wheat cropping system increases wheat production. *Indian Farming* **52,** 3–6.
Setter, T. L., Laureles, E. V., and Mazaredo, A. M. (1997). Lodging reduces yield of rice by self-shading and reductions in canopy photosynthesis. *Field Crops Res.* **49,** 95–106.
Setyanto, P., Makarim, A. K., Fagi, A. M., Wassmann, R., and Buendia, L. V. (2000). Crop management affecting methane emissions from irrigated and rainfed rice in Central Java (Indonesia). *Nutr. Cycl. Agroecosyst.* **58,** 85–93.
Shah, T. (2005). Groundwater and human development: Challenges and opportunities in livelihoods and environment. *Water Sci. Technol.* **51,** 27–37.
Shah, T., Bruke, J., Vullholth, K., Angelica, M., Custodio, E., Daibes, F., Hoogesteger van Dijk, J. D., Giordano, M., Girman, J., van der Gun, J., Kendy, E., Kijne, J., *et al.* (2007). Groundwater: A global assessment of scale and significance. *In* "Water for Food, Water for Life: A Comprehensive Assessment of Water Management in Agriculture" (D. Molden, Ed.). Earthscan, London.
Sharma, P. K., Ladha, J. K., and Bhushan, L. (2003). Soil physical effects of puddling in rice-wheat cropping systems. *In* "Improving the Productivity and Sustainability of Rice-Wheat Systems: Issues and Impacts" (J. K. Ladha, J. E. Hill, J. M. Duxbury, R. K. Gupta, and R. J. Buresh, Eds.), pp. 97–113. ASA, CSSA, SSSA, Madison, WI, ASA Special Publication 65.

Sharma, P. K., and De Datta, S. K. (1985). Effect of puddling on soil physical properties and processes. *In* "Soil Physics and Rice", pp. 217–234. International Rice Research Institute, Los Baños, Philippines.
Sharma, P. K., and De Datta, S. K. (1986). Physical properties and processes of puddled rice soils. *Adv. Soil Sci.* **5,** 139–178.
Sharma, P. K., Bhushan, L., Ladha, J. K., Naresh, R. K., Gupta, R. K., Balasubramanian, B. V., and Bouman, B. A. M. (2002). Crop-water relations in rice-wheat cropping under different tillage systems and water-management practices in a marginally sodic medium-textured soil. *In* "Water-Wise Rice Production" (B. A. M. Bouman, H. Hengsdijk, B. Hardy, P. S. Bindraban, T. P. Tuong, and J. K. Ladha, Eds.), pp. 223–235. International Rice Research Institute, Los Baños, Philippines.
Sharma, P., Tripathi, R. P., and Singh, S. (2005a). Tillage effects on soil physical properties and performance of rice-wheat cropping system under shallow water table conditions of Tarai, Northern India. *Eur. J. Agron.* **23,** 327–335.
Sharma, P., Tripathi, R. P., Singh, S., and Kumar, R. (2004). Effects of tillage on soil physical properties and crop performance under rice-wheat system. *J. Indian Soc. Soil Sci.* **52,** 12–16.
Sharma, R. K., Chhokar, R. S., Jat, M. L., Singh, Samar, Mishra, B., and Gupta, R. K. (2008). Direct drilling of wheat into rice residues: Experiences in Haryana and western Uttar Pradesh. *In* "Permanent Beds and Rice-Residue Management for Rice-Wheat Systems in the Indo-Gangetic Plain". (E. Humphreys and C. H. Roth, Eds.), Proceedings of a workshop held in Ludhiana, India, 7–9 September 2006. ACIAR Proceedings No. 127, pp. 147–158. Australian Centre for International Agricultural Research, Canberra, Australia, www.aciar.gov.au/publication/term/18.
Sharma, S. K. (1984). Principles and Practices of Irrigation. Oxford and IBH Publication Co., New York.
Sharma, S. K., Pandey, D. K., Gangwar, K. S., and Tomar, O. K. (2005b). Effect of crop establishment methods on performance of rice (*Oryza sativa*) cultivars and their effects on succeeding wheat (*Triticum aestivum*). *Indian J Agron.* **50,** 253–255.
Sharma, S. K., Tomar, R. K., and Gangwar, K. S. (1995). Effects of crop establishment and tillage practices on yield and economics of irrigated rice (*Oryza sativa*)–wheat (*Triticum aestivum*) system. *Indian J. Agric. Sci.* **65,** 636–638.
Shaviv, A., and Mikkelsen, R. L. (1993). Controlled-release fertilizers to increase efficiency of nutrient use and minimize environmental degradation: A review. *Fert. Res.* **35,** 1–12.
Shoji, S., Delgado, J., Mosier, A., and Miura, Y. (2001). Use of controlled release fertilizers and nitrification inhibitors to increase nitrogen use efficiency and to conserve air and water quality. *Commun. Soil Sci. Plant Anal.* **32,** 1051–1070.
Shukla, A. K., Ladha, J. K., Singh, V. K., Dwivedi, B. S., Balasubramanian, V., Gupta, R. K., Sharma, S. K., Singh, Y., Pathak, H., Pandey, P. S., Padre, A. T., and Yadav, R. L. (2004). Calibrating the leaf color chart for nitrogen management in different genotypes of rice and wheat in a systems perspective. *Agron. J.* **96,** 1606–1621.
Sidhu, H. S., Singh, Manpreet, Blackwell, J., Humphreys, E., Bector, V., Singh, Yadvinder, Singh, Malkeet, and Singh, Sarbjit (2008). Development of the Happy Seeder for direct drilling into combine harvested rice. *In* "Permanent Beds and Rice-Residue Management for Rice-Wheat Systems in the Indo-Gangetic Plain". (E. Humphreys and C. H. Roth, Eds.), Proceedings of a Workshop Held in Ludhiana, India, 7–9 September 2006. ACIAR Proceedings No. 127, pp. 159–170. Australian Centre for International Agricultural Research, Canberra, Australia, www.aciar.gov.au/publication/term/18.
Singh, A. K., Choudhury, B. U., and Bouman, B. A. M. (2002a). Evects of rice establishment methods on crop performance, water use, and mineral nitrogen. *In* "Water-Wise Rice Production" (B. A. M. Bouman, H. Hengsdijk, B. Hardy, P. S. Bindraban, T. P. Tuong, and J. K. Ladha, Eds.), pp. 237–246. International Rice Research Institute, Los Baños, Philippines.

Singh, D. K., and Singh, A. K. (2002). Groundwater situation in India: Problems and perspective. *Water Res. Dev.* **18,** 563–580.
Singh, G. (2008). Integrated weed management in direct-seeded rice. *In* "Direct Seeding of Rice and Weed Management in the Irrigated Rice-Wheat Cropping System of the Indo-Gangetic Plains" (Y. Singh, V. P. Singh, B. Chauhan, A. Orr, A. M. Mortimer, D. E. Johnson, and B. Hardy, Eds.), pp. 161–176. International Rice Research Institute, Los Baños, Philippines and Directorate of Experiment Station, G.B. Pant University of Agriculture and Technology, Pantnagar, India.
Singh, G. R., and Singh, T. A. (1988). Leaching losses and use efficiency of nitrogen in rice fertilized with urea supergranules. *J. Ind. Soc. Soil Sci.* **36,** 274–279.
Singh, G., Singh, R. K., Singh, V. P., Singh, B. B., and Nayak, R. (1999). Effect of crop weed competition on yield and nutrient uptake by direct seeded rice (*Oryza sativa*) in rainfed lowland situation. *Indian J. Agron.* **44,** 722–727.
Singh, Samar, Ladha, J. K., Gupta, R. K., Bhushan, L., Rao, A. N., Sivaprasad, B., and Singh, P. P. (2007). Evaluation of mulching, intercropping with Sesbania and herbicide use for weed management in dry-seeded rice (*Oryza sativa* L.). *Crop Prot.* **26,** 518–524.
Singh, S. K., Bharadwaj, V., Thakur, T. C., Pachauri, S. P., Singh, P. P., and Mishra, A. K. (2009a). Influence of crop establishment methods on methane emission from rice fields. *Curr. Sci.* **97,** 84–89.
Singh, S., and Singh, G. (2007). Evaluation of different methods of establishment in wheat (*Triticum aestivum*) after different methods of rice (*Oryza sativa*) establishment. *Pantnagar J. Res.* **5,** 36–40.
Singh, S., Bhushan, L., Ladha, J. K., Gupta, R. K., Rao, A. N., and Sivaprasad, B. (2006). Weed management in dry-seeded rice (*Oryza sativa*) cultivated in the furrow-irrigated raised-bed planting system. *Crop Prot.* **25,** 487–495.
Singh, S., Sharma, R. K., Gupta, R. K., and Singh, S. S. (2008). Changes in rice-wheat production technologies and how rice-wheat became a success story: Lessons from zero-tillage wheat. *In* "Direct-Seeding of Rice and Weed Management in the Irrigated Rice-Wheat Cropping System of the Indo-Gangetic Plains" (Y. Singh, V. P. Singh, B. Chauhan, A. Orr, A. M. Mortimer, D. E. Johnson, and B. Hardy, Eds.), pp. 91–106. International Rice Research Institute, Los Baños, Philippines and Directorate of Experiment Station, G.B. Pant University of Agriculture and Technology, Pantnagar, India.
Singh, S., Sharma, S. N., and Prasad, R. (2001). The effect of seeding and tillage methods on productivity of rice-wheat cropping system. *Soil Till. Res.* **61,** 125–131.
Singh, S., Tripathi, R. P., Sharma, P., and Kumar, R. (2004). Effect of tillage on root growth, crop performance and economics of rice (*Oryza sativa*)–wheat (*Triticum aestivum*) system. *Indian J. Agric. Sci.* **74,** 300–304.
Singh, Samar, Chhokar, R. S., Gopal, R., Ladha, J. K., Gupta, R. K., Kumar, V., and Singh, M. (2009b). Integrated weed management: A key to success for direct-seeded rice in the Indo-Gangetic Plains. *In* "Integrated Crop and Resource Management in the Rice-Wheat System of South Asia" (J. K. Ladha, Y. Singh, O. Erenstein, and B. Hardy, Eds.), pp. 261–278. International Rice Research Institute, Los Baños, Philippines.
Singh, U. P., Singh, Y., Kumar, V., and Ladha, J. K. (2009c). valuation and promotion of resource-conserving tillage and crop establishment techniques in the rice-wheat system of eastern India. *In* "Integrated Crop and Resource Management in the Rice-Wheat System of South Asia" (J. K. Ladha, Y. Singh, O. Erenstein, and B. Hardy, Eds.), pp. 151–176. International Rice Research Institute, Los Baños, Philippines.
Singh, Y., Bhardwaj, A. K., Singh, S. P., Singh, R. K., Chaudhary, D. C., Saxena, A., Singh, V., Singh, S. P., and Kumar, A. (2002b). Effect of rice (*Oryza sativa*) establishment methods, tillage practices in wheat (*Triticum aestivum*) and fertilization on soil physical properties and rice–wheat system productivity on a silty clay Mollisol of Uttaranchal. *Indian J. Agric. Sci.* **72,** 200–205.

Singh, Y., Singh, G., Johnson, D., and Mortimer, M. (2005). Changing from transplanted rice to direct seeding in the rice-wheat cropping system in India. *In* "Rice Is Life: Scientific Perspectives for the 21st Century" (K. Toriyama, K. L. Heong, and B. Hardy, Eds.), pp. 198–201. International Rice Research Institute, Los Baños, Philippines and Japan International Research Center for Agricultural Sciences, Tsukuba, Japan.

Slaton, N. A., (Ed.) (2001). *In* "Rice Production Handbook—MP192". University of Arkansas, Division of Agriculture, Cooperative Extension Service, Little Rock, Arkansas, USA.

Smith, R. J. Jr. (1988). Weed thresholds in Southern US rice, *Oryza sativa. Weed Technol.* **3,** 414–419.

Snyder, C. S., and Slaton, N. A. (2001). Rice production in the United States: An overview. *Better Crops* **85,** 3–7.

So, H. B., and Woodhead, T. (1987). Alleviation of soil physical limits to productivity of legumes in Asia. *In* "ACIAR Proceedings on Food Legume Improvement for Asian Farming systems" (E. S. Wallis and D. E. Byth, Eds.), pp. 112–120. No. 18, Australian Centre for International Agricultural Research, Canberra, Australia.

Soejima, H., Sugiyama, T., and Ishihara, K. (1992). Changes in cytokinin activities and mass spectrometric analysis of cytokinins in root exudates of rice plant (*Oryza sativa* L.). *Plant Physiol.* **100,** 1724–1729.

Soejima, H., Sugiyama, T., and Ishihara, K. (1995). Changes in the chlorophyll contents of leaves and in levels of cytokinins in root exudates during ripening of rice cultivars Nipponbare and Akenohoshi. *Plant Cell Physiol.* **36,** 1105–1114.

Song, C., Sheng-guan, C., Xin, C., and Guo-ping, Z. (2009). Genotypic differences in growth and physiological responses to transplanting and direct seeding cultivation in rice. *Rice Sci.* **16,** 143–150.

Spanu, A. G., Andria, P. R., Lavini, A., and Chiranda, F. Q. (1996). Yield response of rice to increasing sprinkler irrigation. *J. Int. Commun. Drain.* **45,** 56–66.

Stangel, P. J., Savant, N. K., and Byrnes, B. H. (1991). Potential of modified ureas for rice. *In* "International Symposium on Rice Research: New Frontiers", November 1990, Hyderabad, India.

State Hydrological Institute (2009). International Hydrological Programme, UNESCO's Intergovernmental Scientific Program in Water Resources, http://webworld.unesco.org/water/ihp/db/shiklomanov/(accessed 25 November 2009).

Stutterheim, N. C., Barbier, J. M., and Nougaredes, B. (1994). The efficiency of fertilizer nitrogen in irrigated, direct seeded rice (*O. sativa* L.) in Europe. *Fert. Res.* **37,** 235–244.

Sudhir-Yadav, Gill, M. S., and Kukal, S. S. (2007). Performance of direct-seeded basmati rice in loamy sand in semi-arid sub-tropical India. *Soil Till. Res.* **97,** 229–238.

Sudhir-Yadav, Gurjeet, Gill, Humphreys, E., Kukal, S. S., and Walia, U. S. (2011a). Effect of water management on dry seeded and puddled transplanted rice. Part 1. Crop performance. *Field Crops Res.* **120,** 112–122.

Sudhir-Yadav, Humphreys, E., Kukal, S. S., Gurjeet, Gill, and Rangarajan, R. (2011b). Effect of water management on dry seeded and puddled transplanted rice. Part 2. Water balance and water productivity. *Field Crops Res.* **120,** 123–132.

Sumita, T., and Ando, M. (2001). Economy of direct seeding of rice in Northeast Thailand and its future direction. *In* "JIRCAS Working Report No. 30," pp. 147–149.

Sur, H. S., Prihar, S. S., and Jalota, S. K. (1981). Effect of rice–wheat and maize–wheat rotations on water transmission and wheat root development in a sandy loam soil of Punjab. *India Soil Till. Res.* **1,** 361–371.

Tabbal, D. F., Bouman, B. A. M., Bhuiyan, S. I., Sibayan, E. B., and Sattar, M. A. (2002). On-farm strategies for reducing water input in irrigated rice: Case studies in the Philippines. *Agric. Water Manage.* **56,** 93–112.

Hongbin, Tao, Brueck, H., Dittert, K., Kreye, C., Lin, S., and Sattelmacher, B. (2006). Growth and yield formation of rice (*Oryza sativa* L.) in the water-saving ground cover rice production system (GCRPS). *Field Crops Res.* **95,** 1–12.

Thakur, A. K., Roychowdhury, S., Kundu, D. K., and Singh, R. (2004). Evaluation of planting methods in irrigated rice. *Arch. Agron. Soil Sci.* **50,** 631–640.

Tisch, S. J., and Paris, T. R. (1994). Labor substitution in Philippines rice farming systems: An analysis of gender work roles. *Rural Sociol.* **59,** 497–514.

Tomar, R. K., Gangwar, K. S., Singh, D., Garg, R. N., Dwivedi, B. S., Gupta, V. K., Sahoo, R. N., Chakraborty, D., and Moharir, A. V. (2005). Effect of tillage systems and moisture regimes on weed growth and productivity of rice-wheat sequence in Inceptisols of Trans Indo-Gangetic Plains. *Ann. Plant Prot. Sci.* **13,** 205–211.

Tomita, S., Nawata, E., Kono, Y., Nagata, Y., Noichana, C., Sributta, A., and Inamura, T. (2003). Differences in weed vegetation in response to cultivating methods and water conditions in rainfed paddy fields in northeast Thailand. *Weed Biol. Manage.* **3,** 117–127.

Tripathi, R. P. (1990). Water requirement in rice-wheat system. *In* "Rice-Wheat Workshop", 15–16 October, Modipuram, Uttar Pradesh.

Tripathi, R. P. (2002). Evaluation and modeling of soil physical properties for optimizing tillage in rice-wheat system. Final Technical Report of ICAR ad hoc Project. G.B. Pant University of Agriculture and Technology, Pantnagar, India, 40pp.

Tripathi, R. P., Sharma, P., and Singh, S. (2005a). Tillage index: An approach to optimize tillage in rice-wheat system. *Soil Till. Res.* **80,** 125–137.

Tripathi, S. C., and Chauhan, D. S. (2001). Effect of tillage and fertilizer on productivity of wheat (*Triticum aestivum*) under dry seeded and transplanted rice conditions. *Indian J. Agron.* **46,** 107–111.

Tripathi, S. C., Mongia, A. D., and Shoran, J. (2005b). Resource conservation technologies and cropping system diversification opportunities. *In* "Conservation Agriculture: Status and Prospects" (I. P. Abrol, R. K. Gupta, and R. K. Malik, Eds.), pp. 72–78. Centre for Advancement of Sustainable Agriculture, New Delhi.

Tripathi, S. C., Nagarajan, S., and Chauhan, D. S. (1999). Evaluation of zero tillage in wheat under different methods of rice *(Oryza sativa*) transplanting. *Indian J. Agron.* **44,** 219–222.

Tsuruta, H. (2002). Methane and nitrous oxide emissions from rice paddy fields. *In* "Proceedings 17th World Congress of Soil Sciences (WCSS), 14–21 August 2002. Bangkok, Thailand", pp. 2100-1–2100-10. http://www.sfst.org/Proceedings/17WCSS_CD/papers/2100.pdf (Assessed on 12 May 2010).

Tuong, T. P. (1999). Productive water use in rice production: Opportunities and limitations. *J. Crop Prod.* **2,** 241–264.

Tuong, T. P., and Bouman, B. A. M. (2003). Rice production in water scarce environments. *In* "Water Productivity in Agriculture: Limits and Opportunities for Improvement" (J. W. Kijne, R. Barker, and D. Molden, Eds.), pp. 53–67. CABI Publishing, Wallingford, UK.

Tuong, T. P., Bouman, B. A. M., and Mortimer, M. (2004). More rice, less water—Integrated approaches for increasing water productivity in irrigated rice-based systems in Asia. *In* "Proceedings of the 4th International Crop Science Congress, 26 September–1 October 2004, Brisbane, Australia on "New directions for a diverse planet", www.cropscience.org.au.

Tuong, T. P., Bouman, B. A. M., and Mortimer, M. (2005). More rice, less water—Integrated approaches for increasing water productivity in irrigated rice-based systems in Asia. *Plant Prod. Sci.* **8,** 231–241.

Tuong, T. P., Singh, A. K., Siopongco, J. D. L. C., and Wade, L. J. (2000). Constraints to high yield of dry-seeded rice in the rainy season of a humid tropic environment. *Plant Prod. Sci.* **3,** 164–172.

Tuong, T. P., Wopereis, M. C. S., Marquez, J. A., and Kropff, M. J. (1994). Mechanisms and control of percolation losses in irrigated puddled rice fields. *Soil Sci. Soc. Am. J.* **58,** 1794–1803.

Turner, F. T., Chen, C. C., and Bollich, C. N. (1982). Coleoptile and mesocotyl length in semi-dwarf rice seedlings. *Crop Sci.* **22,** 43–46.

UC Irvine (University of California—Irvine (2009). Satellites unlock secret to northern India's vanishing water. *Science Daily*, www.sciencedaily.com/releases/2009/08/090812143938.htm(accessed 13 October 2009).

Varade, S. B. (1990). Establishment of non-rice crops in previously puddled soils. *In* "Transactions of the 14th International Congress of Soil Science, Kyoto," vol. I, pp. 170–174.

von Braun, J., and Bos, M. S. (2004). The changing economics and politics of rice: Implications for food security, globalization, and environmental sustainability. *In* "Rice Is Life: Scientific Perspectives for the 21st Century" (K. Toriyama, K. L. Heong, and B. Hardy, Eds.), pp. 7–20. International Rice Research Institute, Los Baños, Philippines and Japan International Research Center for Agricultural Sciences, Tsukuba, Japan.

Vongsaroj, P. (2000). Wild and weedy rice in Thailand. *In* "Wild and Weedy Rice in Rice Ecosystems in Asia: A Review" (B. B. Baki, D. V. Chin, and M. Mortimer, Eds.), pp. 55–57. Limited Proceedings No. 2. IRRI, Los Baños, Philippines.

Vongsaroj, P. (1997). Weed Management in Paddy Fields. Botany and Weed Science Division, Department of Agriculture, Bangkok Amarin Printing Company, Bangkok Thailand, 175 p.

Wada, G. (1969). The effect of nitrogenous nutrition on the yield-determining process of rice plant. *Bull. Natl. Inst. Agric. Sci. Ser. A* **16,** 27–167 (in Japanese with English summary).

Wah, Cheong Ah. (1998). Direct-seeded rice in Malaysia: A success story. APAARI Publication 1998/1, 33 p.

Wang, B., Xu, Y., Wang, Z., Li, Z., Guo, Y., Shao, K., and Chen, Z. (1999). Methane emissions from rice fields affected by organic amendment, water regime, crop establishment, and rice cultivar. *Environ. Monit. Assess.* **57,** 213–228.

Wang, Z., Delaune, R. D., Masscheleyn, P. H., and Patrick, W. H. Jr. (1993). Soil redox and pH effects on methane production in a flooded rice soil. *Soil Sci. Soc. Am. J.* **57,** 382–385.

Wassmann, R., Neue, H. U., Ladha, J. K., and Aulakh, M. S. (2004). Mitigating greenhouse gas emissions from rice–wheat cropping system in Asia. *Environ. Dev. Sustain.* **6,** 65–90.

Watanabe, H., Azmi, M., and Zuki Md, I. (1996). Ecology of Major Weeds and Their Control in Direct-Seeded Rice Culture of Malaysia. MARDI/MADA/JIRCAS Collaborative Study (1992–96). Malaysian Agricultural Research and Development Institute, Serdang (Malaysia), 202 pp.

Way, M. O., and Cockrell, J., (Eds.) (2008). *In* "2008 Texas rice production guidelines". AgriLife Ext. Ser. B-6131, 2–08.

Weerakoon, W. M. W., Mutunayake, M. M. P., Bandara, C., Rao, A. N., Bhandari, D. C., and Ladha, J. K. (2011). Direct-seeded rice culture in Sri Lanka. *Field Crops Res.* **121,** 53–63.

Wells, B. R., and Faw, W. F. (1978). Short-statured rice response to seeding and N rates. *Agron. J.* **70,** 477–480.

Wells, B. R., and Norman, R. J. (1992). Response of rice to amended ureas as nitrogen sources. *In* "Arkansas Soil Fertility Studies 1991" (W. E. Sabbe, Ed.), Res. Ser. 421, pp. 65–69. Ark. Agric. Exp. Stn, Fayetteville.

Weston, L. A. (1996). Utilization of allelopathy for weed management in agroecosystems. *Agron. J.* **88,** 860–866.

Williams, J. F., and Peterson, M. L. (1973). Relations between alpha-amylase activity and growth of rice seedlings. *Crop Sci.* **13,** 612–615.

Wilson, C. E. Jr., Norman, R. J., and Wells, B. R. (1990). Dicyandiamide influence on uptake of preplant-applied fertilizer nitrogen by rice. *Soil Sci. Soc. Am. J.* **54,** 1157–1161.

Wong, H. S., and Morooka, Y. (1996). Economy of direct seeding rice farming. "Recent Advances in Malaysian Rice Production", pp. 275–287. Malaysia: Muda Agricultural Development Authority and Japan: Japan International Research Center for Agricultural Sciences.

Woodhead, T. (1990). Problems and opportunities in soil physical management in rice-based cropping systems. *In* "Transactions of International Congress of Soil Science, Kyoto," vol. I, pp. 140–145.

Wu, Y., Xin He, P., Chang Hui, Ge, Xun, S. Jian, Liang, S., and Wan Gen, K. (2008). A study on suitable sowing date and sowing rate of a new late japonica hybrid rice combination Bayou 52 in no-tillage and direct seeding cultivation. *Hybrid Rice* **23,** 48–50.

Xia, J., and Chen, Y. Q. D. (2001). Water problems and opportunities in hydrological sciences in China. *Hydrol. Sci. J.* **46,** 907–922.

Xie, G. H., Yu, J., Wang, H., and Bouman, B. A. M. (2008). Progress and yield bottleneck of aerobic rice for the North China Plains: A case study of varieties Handao 297 and Handao 502. *Agric. Sci. China* **7,** 641–646.

Yaduraju, N. T., and Mishra, J. S. (2004). Weed management in rice with special orientation to export. "SAARC RICE EXPO 2004", pp. 111–115. Maharashtra Chamber of Commerce and Industry and Rice Exporters Association of Pakistan, Mumbai, India.

Yaduraju, N. T., and Mishra, J. S. (2005). Sedges in rice culture and their management. *In* "Direct Seeding of Rice and Weed Management in the Irrigated Rice-Wheat Cropping System of the Indo-Gangetic Plains" (Y. Singh, G. Singh, V. P. Singh, P. Singh, B. Hardy, D. E. Johnson, and M. Mortimer, Eds.), p. 17. Directorate of Experiment Station, G.B. Pant University of Agriculture and Technology, Pantnagar, India.

Yadvinder-Singh, Brar, N. K., Humphreys, E., Singh, Bijay, and Timsina, J. (2008). Yield and nitrogen use efficiency of permanent bed rice-wheat systems in northwest India: Effect of N fertilization, mulching and crop establishment method. *In* "Permanent Beds and Rice-Residue Management for Rice-Wheat Systems in the Indo-Gangetic Plain. ACIAR Proceedings No. 127" (E. Humphreys and C. H. Roth, Eds.), Australian Centre for International Agricultural Research, Canberra, Australia, www.aciar.gov.au/publication/term/18 pp. 62–78 (accessed 17 November 2008).

Yoshida, S. (1981). Fundamentals of Rice Crop Science. International Rice Research Institute, Manila, Philippines.

Yoshinaga, S. (2005). Direct-seeding cultivation of rice in Japan: Stabilization of seedling establishment and improvement of lodging resistance. *In* "Rice Is Life: Scientific Perspectives for the 21st Century, 4–7 November 2004. Proceedings of the World Rice Research Conference" (K. Toriyama, K. L. Heong, and B. Hardy, Eds.), pp. 184–186. Japan International Research Center for Agricultural Sciences, Tsukuba, Japan and International Rice Research Institute, Los Baños, Philippines.

Yoshinaga, S., Wakimoto, K., Tasaka, K., Matsushima, K., Togashi, T., and Shimotsubo, K. (2001). Growth characteristics of submerged hill-seeded rice (*Oryza sativa* L.) in warmer regions of Japan: Differences of growth as compared with broadcast-seeded rice. *Jpn. J. Crop Sci.* **70,** 541–547.

Yun, Y. D., Park, T. S., and Oh, Y. J. (1993). Establishment of Dry Seeding Cultivation Method. Annual Research Report 1993 (Rice) National Crop Experiment Station, Suwon, Korea.

Zhao, D. L., Atlin, G. N., Bastiaans, L., and Spiertz, J. H. J. (2006). Cultivar weed competitiveness in aerobic rice: Heritability, correlated traits, and the potential for indirect selection in weed-free environment. *Crop Sci.* **46,** 372–380.

Zwart, S. J., and Bastiaanssen, W. G. M. (2004). Review of measured crop water productivity values for irrigated wheat, rice, cotton and maize. *Agric. Water Manage.* **69,** 115–133.

Index

W

Z